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Therapeutic potential of nanocurcumin and black seed (*Nigella sativa*) in *Escherichia coli* LPS-induced joint inflammation in pigeons

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Abstract

This study comparatively evaluated the protective effects of nanocurcumin and black seed against bacterial lipopolysaccharide (LPS)-induced acute arthritis in pigeons, compared with the standard nonsteroidal anti-inflammatory drug diclofenac. Thirty-five pigeons were divided into five groups: sham control (T1), LPS-induced arthritis control (T2), and treatment groups receiving diclofenac (T3), black seed (T4), or nanocurcumin (T5) after LPS challenge. Arthritis was induced by intra-articular LPS injection into the right hock joint. At 48 hours post-injection, synovial fluid was collected for pro-inflammatory cytokine measurement, serum epinephrine and corticosterone levels were assayed, and joint tissue was histopathologically evaluated. LPS injection (T2) significantly increased synovial pathology severity (hyperplasia, inflammation) versus the sham group, with congestion, reactive fibrosis, mononuclear inflammatory cell aggregation, dense adipocyte accumulation, and chondrocyte hypertrophy. Group T4 showed a severe inflammatory reaction, while groups T3 and T5 had a total score of 6. Systemically, LPS significantly elevated serum epinephrine and corticosterone; diclofenac produced the greatest reduction in both compared with the natural supplements. In synovial fluid, the positive control had the highest tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) concentrations. Diclofenac showed the strongest TNF-α suppression, while both black seed and nanocurcumin significantly reduced TNF-α and IL-6 versus the untreated group. Nanocurcumin significantly reduced corticosterone compared with black seed. Diclofenac exhibited the most potent anti-inflammatory effect; however, both nanocurcumin and black seed provided significant therapeutic benefits in mitigating LPS-induced acute arthritis, suggesting their potential as viable natural adjuncts in avian inflammatory disease management, particularly given the toxicity profile of nonsteroidal anti-inflammatory drugs.

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Introduction

Inflammation in birds, like in other animals, involves a complex biological response to harmful stimuli, characterized by redness, swelling, heat, pain and loss of function. It can manifest in various forms such as airsacculitis, pododermatitis, arthritis and can be triggered by infections, injuries, or other underlying health issues.[1] Lipopolysaccharide (LPS; also known as endotoxin), a crucial component of the cell wall in gram-negative bacteria, serves as a powerful trigger for inflammation. It activates inflammatory processes through Toll-like receptor 4, which is present on the surface of leukocytes, including monocytes/macrophages and heterophils.[2] This ligand-receptor interaction triggers macrophages to produce and release vasoactive mediators including cytokines like interleukin (IL)-1b, IL-6, tumor necrosis factor-alpha (TNF-α) and chemokines such as IL-8.[3]

Nonsteroidal anti-inflammatory drugs, known as nonsteroidal anti-inflammatory drugs (NSAIDs), are commonly used to reduce pain and manage inflammation. These drugs are also used to combat conditions such as LPS endotoxemia, which involves toxins in the blood. Their widespread use makes them a key option for controlling pain and swelling in many medical situations.[4] These drugs are commonly used in different animal species and have shown promising results in treating many inflammatory responses. However, they also carry severe

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side effects such as: cellular damage, hepatotoxicity and nephrotoxicity of NSAIDs.[5] Due to the notable side effect profiles associated with steroidal and NSAID medications, there is an increasing interest in natural compounds including dietary supplements and herbal remedies which have been utilized for centuries to alleviate pain and inflammation.[6] Numerous natural compounds also function by inhibiting inflammatory pathways in a manner akin to NSAIDs.

Curcumin is a bright yellow compound found in the rhizomes of turmeric (*Curcuma longa* L.). It plays a key role in reducing inflammation by blocking certain enzymes that regulate inflammation, including cyclooxygenase-2, lipoxygenase, inducible nitric oxide synthase, reactive oxygen species, and signal transducer and activator of transcription.[7] These proteins are involved in causing inflammation, when curcumin suppresses their activity, the overall level of inflammation decreases. Nonetheless, the low bioavailability of curcumin presents a significant obstacle to achieving its complete potential.[8] Many methods have been tried to improve its absorption, including formulations of curcumin-like nanocarriers.[9] These approaches aim to deliver more of the compound into body and increase its effectiveness. Black seed (*Nigella sativa* L.), also known as black cumin, is native to Mediterranean countries. Thymoquinone (TQ) is a major phytochemical bioactive ingredient in *N. sativa* oil and extracts.[10] Studies have shown this component has many health benefits such as: anti-inflammatory, anti-cancer, anti-hypertensive, and immunity-boosting effects.[11,12]

To our knowledge, no report is available about the comparative effects of NSAIDs and natural compounds on acute arthritis induced by bacterial LPS in avian species. In the present study protective effects of nanocurcumin and black seed on LPS- induced arthritis in pigeon model were studied and compared to diclofenac.

Materials and Methods

Drugs and chemicals. Lipopolysaccharide from *Escherichia coli* O127:B8 (Sigma-Aldrich, St. Louis, USA); diclofenac from Aburaihan Pharmaceutical Company (Tehran, Iran); nanocurcumin was purchased from Exir Nano Sina Company (Tehran, Iran), and black seed was obtained from Barij Essence (Kashan, Iran).

Animals and experiment design. A total number of 35 clinically healthy pigeons of mixed gender with body weight 270 ± 30.00 g were purchased from a local supplier. Birds were housed in stainless steel cages and mashed feed and water were administered *ad libitum*. Proper light, temperature, and general management conditions were kept for birds. The birds were allowed to get accustomed to this condition for 7 days. Then, birds allocated in five experimental groups (T1-T5) with seven birds in each as follows: T1 (Sham control group): the birds received 0.05 mL of normal saline by intra-articularly injection into the right hock joint; T2 (Positive control: acute arthritis): the birds were injected with LPS (dissolved in 0.90% saline) intra-articularly into the right hock joint at a dose of 1.00 mg kg^{-1} body weight;[13] Arthritis was also induced in the right hock joint of all birds in groups T3-T5 as mentioned above and then different therapeutic approaches were used as follows: T3 (LPS + Diclofenac): the birds were treated by diclofenac at a dose of 10.00 mg kg^{-1} body weight once intramuscularly, T4 (LPS + Black seed): the birds were treated by oral black seed at a dose of 500 mg kg^{-1} twice daily immediately after LPS injection to 48 hr,[14] and T5 (LPS + nanocurcumin): the birds were treated by oral nanocurcumin at a dose of 50.00 mg kg^{-1} twice daily immediately after LPS injection to 48 hr.[15] The test protocol was approved by the Animal Ethics Committee of Amol University of Special Modern Technologies (Approval no: IR. AUSMT. REC. 1403. 28).

Blood collection and hormonal assays. At 48 hr after injection of LPS (End of experiment), blood samples were taken from the brachial veins of all birds in experimental groups for serum separation and rapidly transferred to the laboratory. After centrifugation (3,000 rpm for 10 min at room temperature) serum was harvested. The serum epinephrine level was assessed using the enzyme-linked immunosorbent assay (ELISA) method with the Human Epinephrine/Adrenaline, EPI ELISA Kit as *per* the manufacturer's instructions (SunLong Biotech Co., Hangzho, China). To measure the serum corticosterone concentration, the corticosterone ELISA kit was utilized (Zellbio GmbH, Lonsee, Germany).

Histopathology. At the end of the experiment, two birds from each group were randomly selected and euthanized. Joint tissue samples of 2.00 cm in length were taken, then for decalcification tissue fixed in 4.00% paraformaldehyde for 24 hr, the samples were thoroughly washed under running water. The samples were subsequently decalcified using a 3.00% nitric acid solution. The decalcification steps were performed at 4.00 ˚C with continuous agitation. After decalcification, samples were dehydrated, clarified, and embedded in paraffin before cutting into 5.00-µm-thick sections using a rotary microtome (Diapath, Martinengo, Italy). After mounting on glass slides, the sections were stained with Hematoxylin and Eosin stain. Hematoxylin and Eosin-stained slides were graded for synovial pathological features, synovial lining cells, synovial mesenchymal components, subsynovial inflammation, and synovial fibrosis.[16] We categorized the scoring as synovial cellularity, subsynovial inflammation, and hypertrophy of chondrocytes. Fibrosis, synoviocyte hyperplasia, and significant adipocytes were included in the category of synovial cellularity. The noted pathological features are included in most published scoring methods.[17,18] Microscopic figures are prepared at 40.00, 100 and 400 × magnification.

Synovial lavage and determination of inflammatory cytokine concentrations. The right intertarsal joint from euthanized birds was rinsed with 0.50 mL of normal saline. Then, the synovial fluid was collected and stored at –20.00 ˚C. The levels of TNF-α, and IL-6 in the synovial fluid were determined using ELISA kits purchased from R&D Systems Inc. (Minneapolis, USA).

Statistical analysis. GraphPad Prism (version 9.0; GraphPad software Inc., San Diego, USA) was used to data evaluation. The differences were analyzed by analysis of variance and the mean ± standard deviation was used to compare the means through post hoc testing with Tukey's HSD. Statistical significance was established at $p < 0.05$.

Results

Histopathological findings. Table 1 details histopathological scoring criteria. Pathological findings of the sections including congestion, perivascular inflammation and follicle-like aggregation of mononuclear inflammatory cells, hypertrophy of chondrocytes, reactive fibrosis (Table 2). In T1 group, we have observed congestion. In T2 group, congestion and minimal inflammation was detected. In group T3, congestion, extensive perivascular accumulation of mononuclear inflammatory cells and hypertrophic chondrocytes were recognized. In T4, congestion, perivascular inflammation, lymphoid follicles and hypertrophic chondrocytes were detected. In T5, congestion, minimal perivascular inflammation and hypertrophic chondrocytes in chondroid matrix were detected. Table 2 demonstrates the representative microscopic figures of each treatment group (Figs. 1 and 2).

Serum epinephrine concentrations. The epinephrine assay demonstrated significant variations among the experimental groups (Fig. 3A). The T2 group exhibited a substantial elevation in epinephrine concentration, reaching approximately 340 pg mg^{-1}, which was significantly higher than all other groups ($p < 0.05$). In contrast, the T1 group showed the lowest level of epinephrine (around 130 pg mg^{-1}), indicating a normal baseline without inflammatory stimulation. Administration of black seed and diclofenac to LPS-challenged birds resulted in reductions in epinephrine levels. These two treatments did not differ significantly from each other, but were significantly lower than the T2 group. Administration of nanocurcumin also had a reducing effect on epinephrine levels in the challenged groups, but not as much as administration of diclofenac and black seed.

Table 1. Histopathological scoring criteria after lipopolysaccharide administration.

Histopathological features	Scores	Description
Enlargement of synovial lining cell		**Layers**
	0	1 layer
	1	2 - 3 layers
	2	4 - 5 layers, few multinucleated cells might occur
	3	More than 5 layers with occasional ulceration or multinucleated cells
Density of resident stromal cells + Inflammatory infiltrates = Synovial fibrosis, inflammation, other mesenchymal components		**Cellularity (fibrosis, adipocytes)**
	0	Normal cellularity
	1	Slightly increased cellularity
	2	Moderately increased cellularity with occasional multinucleated cells
	3	Greatly increased cellularity with occasional multinucleated giant cells, pannus formation and rheumatoid granulomas
		Inflammatory infiltrate
	0	No inflammatory infiltrate
	1	Few perivascularly situated lymphocytes or plasma cells
	2	Numerous lymphocytes or plasma cells with formation of follicle-like aggregates
	3	Dense band-like inflammatory infiltrate or numerous large follicle-like aggregates
Hypertrophy of chondrocytes	+	Presence of hypertrophy
	–	Lack of hypertrophy

Table 2. Result of histopathological evaluation of the articular surface, Hematoxylin and Eosin Eeosin sections.

Groups	Hypertrophy of chondrocytes	Synovial fibrosis	Synovial mesenchymal components	Subsynovial inflammation	Synovial lining cells
Sham control	-	0	0	0	0
Positive control	-	0	1	1	0
Lipopolysaccharide + Diclofenac	+	1	1	2	2
Lipopolysaccharide + Black seed	+	0	1	3	1
Lipopolysaccharide + Nanocurcumin	+	1	1	2	2

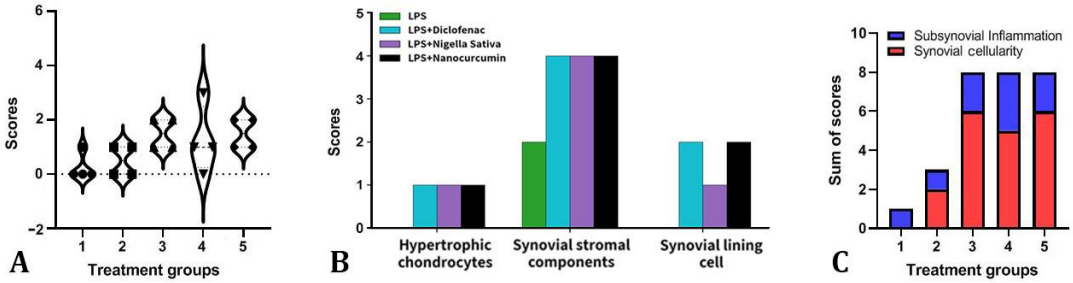


Fig. 1. A) The result of histopathology is presented as violin plot. The violin plot shows distribution of scores of histopathological parameters of Table 2. In addition, it presents comparison of all group-specific data (synovial stromal cell components and subsynovial inflammation) collected in Table 2. **B)** The contingency figure of histopathological data is demonstrated as interleaved bars. The three histopathological features of five groups are included in the Figure. **C)** Stacked columns of synovial cellularity and adjacent inflammation in five treatment groups are indicated. (Treatment groups: 1: sham control, 2: positive control, 3: lipopolysaccharide (LPS) + Diclofenac, 4: LPS + Black seed, 5: LPS + Nanocurcumin).

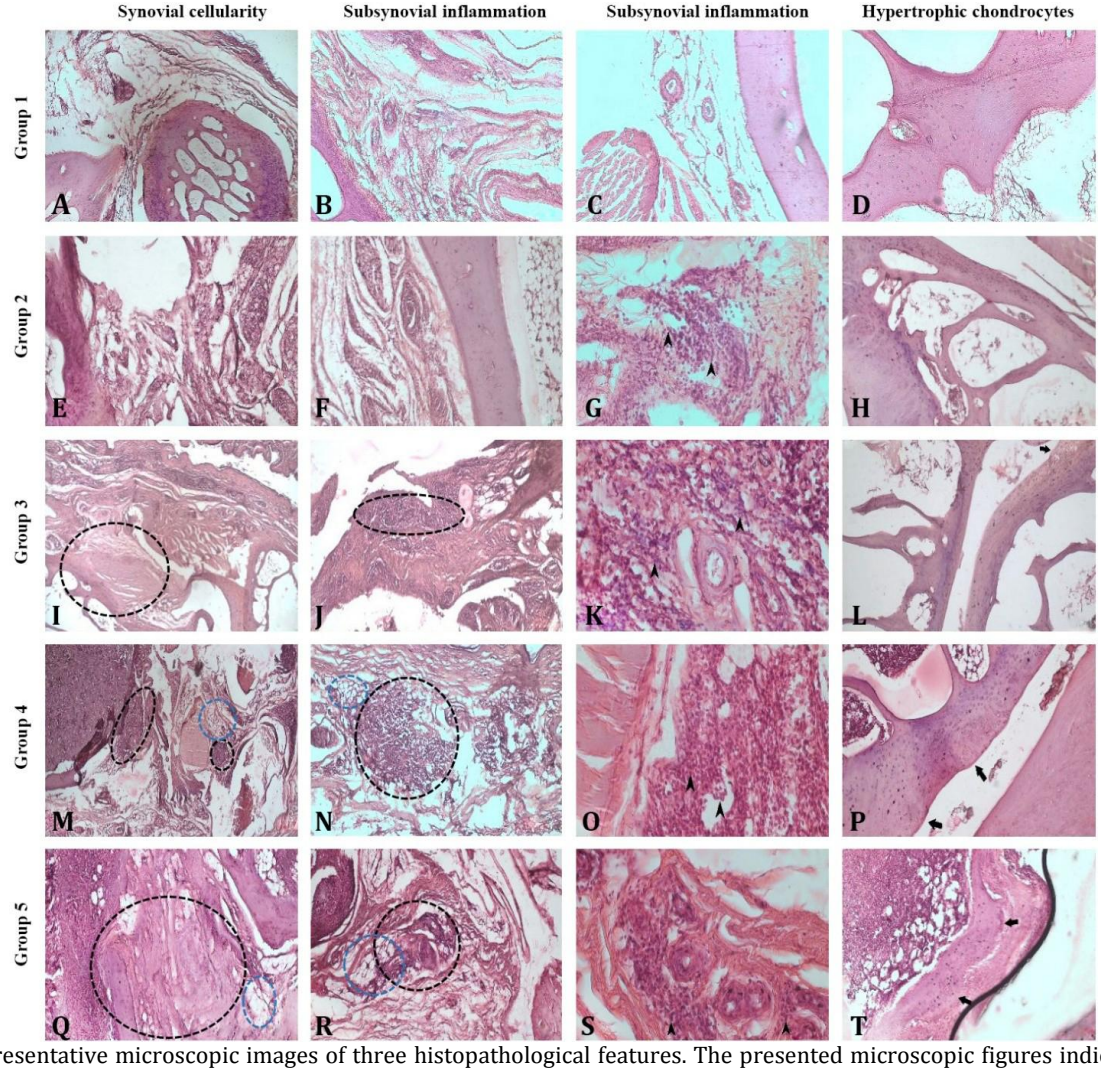


Fig. 2. Representative microscopic images of three histopathological features. The presented microscopic figures indicate various cell populations of synovial sections. A hyperplastic response was recognized in sections which are marked with dashed circle. Black dashes surround fibrosis foci and blue dashes surround adipocyte aggregation. In third column arrowheads point to mononuclear inflammatory cells, lymphocytes and plasma cells. Inflammatory cells are accumulated perivascular or in follicle-like aggregations. In fourth column, arrows point to foci of chondrocyte hypertrophy. Group: 1: sham control, Group 2: positive control, Group 3: lipopolysaccharide (LPS) + Diclofenac, Group 4: LPS + Black seed, Group 5: LPS + Nanocurcumin, (Hematoxylin and Eosin stain A, I, J, M: 40.00×, B, C, D, E, F, H, L, N, P, Q, R, T: 100×, and G, K, O, S: 400×).

Serum corticosterone concentrations. Lipopolysaccharide administration significantly increased the corticosterone levels of the experimental groups compared to sham control group (T1; $p < 0.05$). The positive control group (T2) showed the highest corticosterone levels, confirming the induction of systemic stress. Diclofenac treatment (T3) significantly reduced corticosterone compared to the other traded groups, although the values remained higher than the T1 group. Both black seed and nanocurcumin (T4 and T5) also reduced corticosterone levels, but their reducing effects were less than diclofenac (T3). It is noteworthy that nanocurcumin administration significantly reduced corticosterone levels compared to black seed administration (Fig. 3B).

Level of IL-6 in the synovial fluid. Figure 3C shows IL-6 levels (pg mg^{-1} protein) across different groups. The positive control group (T2) exhibited the highest IL-6 concentration (~147 pg mg^{-1}), while the Sham control (T1) had the lowest (~32.00 pg mg^{-1}). Both black seed and nanocurcumin significantly reduced IL-6 concentrations compared to the untreated LPS group, however, the magnitude of reduction was greater in the diclofenac (T3) and black seed (T4) groups than in the nanocurcumin (T5) group.

Level of TNF-α in the synovial fluid. The comparison of TNF-α levels across experimental groups revealed significant differences ($p < 0.05$). The positive control group (T2) exhibited the highest TNF-α concentration (approximately 135 pg mg^{-1}), confirming the pro-inflammatory effect of LPS. Treatment with black seed and nanocurcumin markedly reduced TNF-α production compared to the positive control, with mean values around 90.00 pg mg^{-1}. However, these reductions were less pronounced than that observed in the diclofenac-treated group, which showed a further decline in TNF-α to nearly 67.00 pg mg^{-1}. As expected, the sham control group (T1) demonstrated the lowest TNF-α level (approximately 35.00 pg mg^{-1}), representing the basal condition without inflammatory stimulation (Fig. 3D).

Discussion

The current study was designed to evaluate the therapeutic efficacy of diclofenac, black seed (*Nigella sativa* L.), and nanocurcumin in a pigeon model of acute arthritis induced by intra-articular LPS injection. Different methods, including infection by *Salmonella Pullorum*, *Staphylococcus aureus*, and *Mycoplasma synoviae*, have been used to induce experimental arthritis in birds.[19-21]

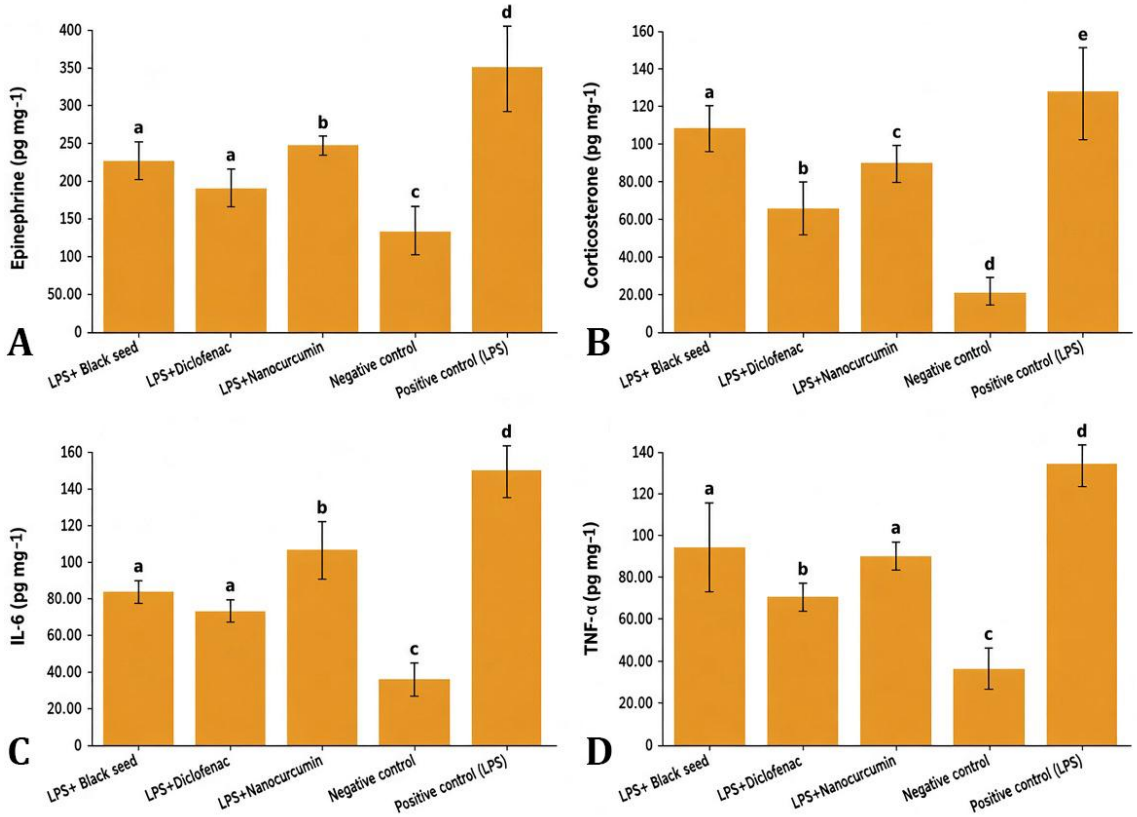


Fig. 3. Effects of treatments on serum **A)** Epinephrine, **B)** Corticosterone, **C)** Interleukin (IL)-6, and **D)** Tumor necrosis factor-alpha (TNF-α) in birds of the different groups. LPS: lipopolysaccharide.
Different letters above bars indicate statistically significant differences among groups ($p < 0.05$).

Various scoring and approaches have been introduced to score synovial damage and osteoarthritis. Although there are no standard approaches, scoring of synovitis, as it is a quantification of synovial change, has been widely used and accepted. Synovial changes include inflammatory, degenerative or traumatic responses, which makes histopathological assessment a controversial method.[17,18] In the current study, we utilized a scoring approach that contains inflammatory and proliferative criteria. Based on histopathological analyses, specimens T3 and T5 exhibited enhanced reparative properties, whereas, specimen 4 demonstrated inflammatory properties (Table 2 and Fig. 2). Histopathological finding of each test sample is indicated in Table 2, and associated violin plot analysis is demonstrated in Figure 1a Each histopathological change is depicted in Figure 2 in a distinct image.

As expected, the sham control group (T1), which received saline only, exhibited no significant synovial changes aside from mild sub-synovial inflammation and vascular congestion, confirming the baseline histological architecture of the pigeon hock joint under non-inflammatory conditions. In contrast, the positive control group (T2) displayed severe synovial hyperplasia, minimal inflammation and evident vascular congestion consistent with acute inflammatory changes elicited by LPS, in previous studies.[22,23]

Among the treatment groups, diclofenac (T3) produced a marked reduction in synovial hyperplasia, fibrosis, and inflammation, confirming its established role as a nonsteroidal anti-inflammatory drug that suppresses prostaglandin synthesis and leukocyte recruitment.[24] However, the presence of extensive perivascular mononuclear infiltration and hypertrophic chondrocytes suggests that diclofenac alone may not fully resolve joint tissue damage in the acute phase.

Black seed treatment (T4) demonstrated moderate cellularity and inflammation, with absent fibrosis. The histological presence of lymphoid follicles and perivascular inflammation highlights the herb immunomodulatory effect rather than a direct anti-inflammatory one. Thymoquinone, the principal bioactive component in *N. sativa*, has been shown to modulate the activity of cytokines like IL-1β and TNF-α and to reduce oxidative stress, though its onset of action may be slower compared to synthetic NSAIDs.[10,11]

The use of nanocurcumin in group T5 produced notable histopathological outcomes in pigeons with LPS-induced acute arthritis. Despite showing elevated scores in synovial hyperplasia and cellularity, indicators of active synovial remodeling, the inflammatory score was significantly lower compared to the other treated groups, especially diclofenac and black seed. Additionally, mild fibrosis and only minimal perivascular inflammation were observed, suggesting a controlled inflammatory environment. These results indicated that while some structural changes remained, nanocurcumin was effective in mitigating active inflammation and possibly promoting early tissue remodeling.

This aligned with growing evidence that nano-curcumin, the nanoparticle form of curcumin, significantly enhanced bioavailability and pharmacodynamic stability compared to unmodified curcumin, which typically exhibited poor absorption and rapid metabolism.[25] Several studies have shown that nanocurcumin accumulates effectively in inflamed tissues and exerts multi-targeted immunomodulatory effects through inhibition of nuclear factor kappa B (NF-κB), mitogen-activated protein kinases and downregulation of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6.[26,27]

Compared to NSAIDs like diclofenac, which act primarily through cyclooxygenase enzyme inhibition and carry risks of gastrointestinal and renal toxicity in long-term use,[28] nanocurcumin exhibits lower toxicity and a broader therapeutic spectrum. While diclofenac-treated birds showed improvements in hyperplasia and fibrosis, they still exhibited extensive perivascular inflammatory infiltration, indicating an incomplete resolution of the immune response. In contrast, nanocurcumin-treated birds displayed minimal perivascular inflammation, suggesting a more regulated immune response.

Interestingly, while synovial hyperplasia and cellularity scores remained high in the nanocurcumin group, this may reflect a remodeling response rather than active pathological inflammation. Nanocurcumin was determined to dampen inflammation in favor of the development of the fibrocellular matrix. Cellular reaction of the nanocurcumin group, excluding inflammation, was higher. It should be noted that nanocurcumin reduced inflammatory response properties compared to other groups (Figs. 1A and 1B).

Previous studies have highlighted curcumin role in promoting cartilage matrix synthesis, increasing chondrocyte viability, and protecting against oxidative damage,[29] which may explain the presence of hypertrophic chondrocytes and chondroid matrix in the histological sections observed in group T5. In summary, the histopathological outcomes in the nanocurcumin group suggest a dual action: effective suppression of acute inflammation and possible promotion of tissue remodeling. Compared to both diclofenac and black seed, nanocurcumin achieved a better balance between inflammation control and tissue preservation. However, the persistence of hyperplasia underscores the need for longer-term studies to determine whether nanocurcumin can fully reverse structural changes or facilitate complete tissue normalization.

Corticosterone and catecholamines, including epinephrine, have been utilized as a humoral indicator of stress in avian species. In this study, administration of LPS resulted in a marked elevation of epinephrine levels,

confirming its well-established role as a potent inducer of systemic inflammatory and stress responses. In contrast, all treatment groups demonstrated a significant reduction in epinephrine, indicating the modulatory effects of the administered therapeutic agents. The greatest decrease in epinephrine was observed in groups 1 and 2, which did not differ significantly from each other; however, both groups showed statistically significant reductions compared with the positive control (LPS) and the nanocurcumin-treated group. In a study investigating the effects of combined anti-inflammatory drugs in pigeons, it was reported that combinations of dexamethasone with piroxicam, meloxicam with dexamethasone and dexamethasone with hydrocortisone exerted the most pronounced reducing effects on epinephrine levels.[30]

Not all inflammatory cell infiltrates have been considered as part of the inflammatory response related to surgical manipulation and postoperative complications in the experiment. Nevertheless, inflammation has been detected in the evaluated sections (Tables 1 and 2). Although inflammation has known to have supportive role in healing, researches have proved that inflammation has detrimental effect on healing process, on the contrary.[31] Various natural (plant-based) and synthetic (Nano-polymers) substances have been trialed as repair materials to suppress inflammation and promote healing.[32,33] In the current study, we have chosen an anti-inflammatory agent, a plant, and nanocurcumin as test materials and used histopathology and statistical analysis to measure healing features and compare the obtained results. Curcumin has been widely used and investigated as a scaffold for the healing of cutaneous defects.[34] Curcumin is known to have anti-inflammatory and anti-oxidant properties.[35] In our research, nanocurcumin was determined to dampen inflammation in favor of the development of the fibrocellular matrix. Cellular reaction of the group 5, excluding inflammation, was higher (Figs. 1B and 1C). It should be noted that nanocurcumin showed enhanced healing properties compared to other groups (Fig. 1C). Hypertrophic chondrocytes are present in cartilage embryonic development. The authors need to recall that this finding could be a disadvantage to cartilage healing. Since it is involved in bone remodeling and ossification.

As shown in Figure 5, LPS stimulation strongly increased IL-6 production compared to the sham control, confirming the inflammatory model. Treatments with black seed and diclofenac effectively reduced IL-6 levels, suggesting anti-inflammatory properties. Diclofenac, as expected from a nonsteroidal anti-inflammatory drug, produced one of the lowest IL-6 responses among the treated groups. Interestingly, black seed also showed a considerable reduction, indicating potential therapeutic benefits as a natural anti-inflammatory agent. In contrast, nanocurcumin treatment did not suppress IL-6 effectively; its levels remained significantly higher than diclofenac and black seed groups, though still lower than the positive control. This may suggest either a weaker efficacy under the tested conditions or variability in response. Overall, the data highlight diclofenac and black seed as potent modulators of IL-6 expression, while nanocurcumin might require different dosing, formulation, or synergistic use with other agents to achieve comparable effects.

The current findings aligned with extensive literature demonstrating that TQ, the key bioactive compound in black seed, dose-dependently inhibits LPS-induced production of TNF-α and other pro-inflammatory mediators (*e.g.*, IL-1β, nitric oxide, prostaglandin E2) via suppression of phosphatidylinositol 3-kinase (PI3K/Akt) and NF-κB signaling pathways in microglial models.[36] Additional *in vitro* and *in vivo* studies corroborate that *N. sativa* L. and TQ reduce NF-κB activation and TNF-α expression while activating the nuclear factor erythroid 2-related factor-2 (Nrf2/ARE) antioxidant pathway.[37] Similarly, nanocurcumin has been shown to significantly attenuate LPS-induced inflammation by improving curcumin bioavailability and targeting inflammatory signaling. In models of LPS-induced mastitis, nanocurcumin effectively reduced TNF-α and IL-1β levels, lipid peroxidation, NF-κB p65 expression and Toll-like receptor 4signaling, while enhancing Nrf2, glutathione and tissue architecture.[38]

These findings confirmed that black seed (via TQ) and nanocurcumin consistently reduced TNF-α and inflammation, through NF-κB inhibition and antioxidant pathway activation and conventional NSAIDs like diclofenac remained more effective for immediate cytokine suppression. Given their favorable safety profiles, black seed and nanocurcumin may serve as viable adjuncts in integrative anti-inflammatory regimens, especially where long-term modulation with lower risk is desired.

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Conflict of interest

The authors declare no competing interest.

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