Anti-inflammatory and Antioxidant Effects of Cardamom Essential Oil-Loaded Nanostructured Lipid Carrier in Rat Osteoarthritis Model

Van Abdulqader Ahmed*, Raz Nawzad Mohemmad*, Basima Sadq Ahmed*, Hemn Hassan Othman

Basic Science Department, College of Pharmacy, University of Sulaimani, Sulaimani, Iraq.

Article Info:

Received 26 Apr 2024
Revised 20 Sept 2024
Accepted 8 Oct 2024
Published 31 Aug 2025
Corresponding Author email:
van.ahmed@univsul.edu.iq
Orcid: https://orcid.org/0009-0003-7412-1411

DOI: https://doi.org/10.32947/ajps.v25i3.1175

Abstract:

Background: Osteoarthritis (OA) is a persistent degenerative arthritis disease defined by the degradation of articular cartilage. Inflammatory mediators release is increased in progressed OA; thereby advocate the cartilage matrix destruction. This study evaluates the use of cardamom essential oil-loaded lipid carrier nanoparticles (CEO-LC NPs) as an antiphlogistic and antioxidant for the management of osteoarthritis induced by monosodium iodoacetate (MIA).

Objectives: The main objective of this research to evaluate the effects of CEO-LC NPs as anti-inflammatory and antioxidant activities, and their possible applications as adjuvants therapies in OA.

Methods: Twenty-one male albino rats were assigned into three groups each 7. OA was induced by articular injection of monosodium iodine acetate (MIA) before the start of the study in the positive control and CEO-LC NPs-treated groups. Then, 0.50 µl of CEO-LC NPs (600 mg) was injected intra-articularly in the CEO-LC NPs-treated group on days 14,18,22, and 26 of the study, while at the same time 0.50 µl of saline was injected in the positive control and negative control groups.

Results: the result indicate significant increase in the serum levels of tumor necrosis factor-α (TNF- α), interleukin-6 (IL-6), interleukin 1β (IL-1β), C-reactive protein, and malondialdehyde (MDA) in positive control group as compared to negative control group. While, treatment of left knee joint with CEO-LC NPs expresses marked decreases in these cytokines, C-rective protein, and malondialdehyde levels.

Conclusions: The study's findings demonstrated that treatment with CEO-LC NPs offers an effective anti-inflammatory and antioxidant effect by reducing cytokines and MDA release and raising the levels of total antioxidant capacity.

Keywords; Anti-inflammatory; Cardamom essential oil nanoparticles, osteoarthritis; pro-inflammatory cytokines; phytochemicals

التأثيرات المضادة للالتهابات ومضادات الأكسدة لزيت الهيل العطري المحمل بالدهون ذات البنية النانوية في نموذج الجرذان لالتهاب المفاصل العظمي

ڤان عبدالقادر احمد*، رەز نوزاد محمد كريم*, باسمة صادق احمد*, هيمن حسن عثمان*
قسم العلوم الاساسية, كلية الصيدلة, جامعة سليمانية, محافظة سليمانية, العراق

الخلاصة

الخلفية: الفصال العظمي المفصلي هو نوع من الامراض التهاب المفاصل التنكسي المزمن يُعرف بإنحلال الغضروف المفصلي. ويزداد إفراز الوسطاء الالتهابات في التهاب المفاصل المتقدم؛ مما يدعم تدميرمصفوفة الغضروف.

الاهداف: تقيّم هذه الدراسة التاثيرات المضادة للالتهاب ومضادة للأكسدة لجسيمات النانوية الحاملة للدهون المحملة بزيت الهيل العطرية (CEO-LC NPs) في علاج الفصال العظمي المفصلي مستحثة.

الطرق: كُلف 21 جرذاً من الذكور جرذ ابيض وتم تقسيمها الي ثلاث مجموعات وكل مجموعة مكونة من سبع جرذان. تم استحاث اللتهاب مفصلي عن طريقة حقن مفصلي للمادة خلات اليود أحادي الصوديوم (MIA) قبل بدء الدراسة في المجموعتين سيطرة الموجبة ومعاملة ب CEO-LC NPs. وبعدها تم حقن مفصلي في المفصل ايسرب 0.50 ميكروملیمترمن CEO-LC NPs بجرعة (٦٠٠ مغ) في المجموعة معاملة ب CEO-LC NPs في الايام ١٤،١٨،٢٢،٢٦ من الدراسة، بينما في نفس الوقت تم حقن 0.50 ميكروملیمترمن المحلول الملحي في المجموعتين سيطرة الموجبة وسيطرة السالبة.

النتائج: اشارت النتائج المناعية الي حدوث ارتفاع معنوي ($p<0.05$) في مستوى تراكيز السيتوكينات TNF-α, IL1, IL6, تركيز الاجهاد التاكسدي مالونالديهيد (MDA), البروتين التفاعلي C, و معايير صورة الدم على حد سواء في المجموعة سيطرة الموجبة مقارنة مع المجموعة سيطرة سالبة كما حصل انخفاضاً معنوياً لمستوى تراكيز هذه السيتوكينات, البروتين التفاعلي C, و MDA في المجموعة معاملة ب CEO-LC NPs بالعلاج قيد الدراسة.

الاستنتاجات: اظهرت نتائج هذه الدراسة ان لمركب CEO-LC NPs تاثيراً مضاداً للالتهاب وللأكسدة من خلال تقليل افراز السيتوكينات وMDA وزيادة مستوى مضادات اكسدة الكلية

الكلمات المفتاحية؛ مضاد للالتهابات؛ زيت الهيل العطري المحمل بالدهون ذات البنية النانوية؛ الفصال العظمي؛ السيتوكينات الداعمة للالتهابات؛ المواد الكيميائية النباتية

Introduction

Osteoarthritis (OA) is a painful, chronic degenerative joint disease and a prevalent manifestation of long-term impairment. OA significantly influence the quality of life by limiting regular daily activities and deterioration of joint function (1). It is characterized by the erosion or destruction of articular cartilage, followed by subsequent synovial membrane inflammation, resulting in pain, swelling, tenderness, and defects in joint articulation. Degenerative disorders like OA are triggered by inflammatory factors, cellular and molecular mechanisms, and metabolic processes throughout their development and progression (2, 3).

The inflammatory response in OA is accompanied by release of abundant inflammatory cytokines, including interleukin IL-1β, tumor necrosis factor (TNF), interferon (INF)-c, IL-6, IL-12, IL-18, and oxidative stress induce nitroso-redox imbalance. The nuclear factor kappa-B (NF-B) and mitogen-activated protein (MAP) kinase pathway are activated, leading to the overexpression of cartilage matrix-degrading enzymes, such as cyclo-oxygenase 2 (COX2). The production of COX-2, IL-6, TNF-a, and NO stimulate the formation of adhesion molecules and the sequestration of leukocytes at the inflammation site, generating tissue damage (4).

The objective of pharmacological management for OA is to mitigate inflammation and reduce pain. This includes the use of non-steroidal and steroidal anti-inflammatory drugs (NSAIDs). Nevertheless, these medications can trigger severe adverse effects, such as dyspepsia, pneumonitis, and abnormalities in the liver function (5).

Recently, there has been a tendency to integrate phytotherapy as effective therapeutic approach worldwide. Medicinal plants have been used to treat various diseases because they contain bioactive compounds with varied biological functions (6).

Cardamom (Elettaria cardamomum), belonging to the family Zingiberaceae, is a perennial herbaceous plant obtained from the seeds and originated in Sri Lanka, southern India, Tanzania and Guatemala (7). Cardamom essential oil (CEO) has found applications in industries such as food, cosmetics, and pharmaceuticals. These characteristics correspond to their diverse constituents of numerous phytochemical compounds, such as phenols, flavonoids, tannins, terpenoids, alkaloids, and sterols (8). CEO exhibits a variety of pharmacological impacts, including antioxidant, anti-inflammatory, anticancer, antifungal, and antimicrobial properties (9).

The goal of nanomedicine is to precisely and efficiently diagnose and treat diseases while minimizing any adverse effects. Due to its effectiveness in encapsulating or attaching medications and other bioactive substances to nanostructures for more precise and controlled delivery to target tissues, nanomedicine has grown in popularity (10). Nanostructured lipid carrier (NLCs), were developed in the latter part of the 1990s (11). NLCs consist of an aqueous phase made up of a surfactant and a mixture of liquid and solid lipids in a ratio of up to 70:30, respectively (12). To minimize toxicity, it is crucial that the lipids utilized in these formulations be compatible with the body (13). High-speed homogenization and ultrasonication are used for the production of NLCs by mixing the aqueous phase, which contains surfactant, and the lipid phase. This approach often yields high-polydispersity nanoparticles, which can be managed with probe-based sonicators (14).

The main objective of this research is to evaluate the impacts of CEO-LC NPs on OA as anti-inflammatory and antioxidant activities, and their possible applications as adjuvant therapies.

Materials and Methods

1. Material

Cardamom essential oil was purchase from mountain rose herbs.com (Sri Lanka) Cocoa butter was purchase from Deluxe Shea butter Co. (Australia) and extra virgin olive oil was purchased from Kasho factory Co. (Sulaimani, Iraq). Tween 80 and DPPH powder were analytical grade and were provided from Merck (Germany).

2. Preparation of CEO-LC NPs:

The low-energy nanoemulsification approach in conjunction with high shear homogenization and sonication was used to prepare CEO-LC NPs. After dissolving the CEO in olive oil, the mixture was added to heated cocoa butter (solid lipid). The warmed aqueous surfactant (Tween 80) solution was then added to the lipid phase drop wise while being homogenized at high shear (Heidolph, Nuremberg, Germany) for 45 minutes at 20,000 rpm. Subsequently, sonication was carried out ten times at one-minute intervals using an ultrasonic processor (Sonics, Vibracell, USA). The ultrasonic processor was configured to run at 0.5 cycles per second at 70% amplitude (200 W, 24 kHz). Throughout these procedures, the suspension's temperature was maintained at 50 ± 5 ^{0}C. Once the heated oil in water

nanoemulsion was created, it underwent cooling to 4 ~°C in a refrigerator to cause the lipid phase to recrystallize, leading to the formation of the NLC. The weight ratio of cardamom essential oil to olive oil in the formula was 1:10, and the remaining variables were as follows: 330 mg of cocoa butter, 500 mg of Tween 80, and 25 ml of purified water (15).

3. Characterization of CEO-LC NPs:

The particle size (z-average size), zeta potential, and the polydispersity index (PDI) of CEO loaded NLCs were determined using Dynamic Light Scattering (DLS) and the zetasizer (Malvern Instruments, U.K.). The surface morphology of obtained NLC was investigated by scanning electron microscopy (KYKY-EM3200). Thermal analysis was performed by differential scanning calorimetry (DSC) using a DSC thermal analyzer (LINSEIS. DSC model P 10.) and X-ray crystallography analysis (15).

4. Animals:

Twenty-one mature male Wistar rats weighing 180-210 grams were used in this experiment. They were kept in well-ventilated rooms that followed a 12-hour light/dark cycle. The rats were housed in sterile cages modified for the laboratory one week before the experiment began, with free access to water and food pellets. The animals were cared for in accordance with guidelines established by the Canadian Council on Animal Care. The Ethics Committee of the College of Pharmacy at Sulaimania University in Iraq certified all handling, utilization, and animal euthanasia procedures used in this investigation. The ethical permission number for this study is PH93-23, April 10, 2023.

5. Induction of Osteoarthritis:

OA was induced by a single intra-articular injection (IAI) of 50 µL monosodium iodoacetate (2 mg) (MIA) dispersed in sterile saline into the left patellofemoral joint of all animals, excluding the negative control group, under anesthesia with a combination of ketamine and xylazine. Negative control animals were administered an equivalent volume of saline. The right knee remained uninjected (16). MIA was obtained from Macklin Biochemical Technology Co. Ltd. (Shanghai, China).

6. Study Design:

The animals were randomly assigned into three groups (n = 7). On the zero day of the experiment, 50 µL of MIA (2 mg) were injected into the left knee joints of the CEO-LC NP treated group and positive control group to induce osteoarthritis, while the negative control group was injected with 50 µL of saline. The CEO-LC NPs treated group received IAIs of CEO-LC NPs at a dose of 600 mg/kg on days 14, 18, 22, and 26, while the negative control and positive control groups received IAIs of saline. CEO-LC NPs doses were based on the previous researches (17). As shown in figure (1)

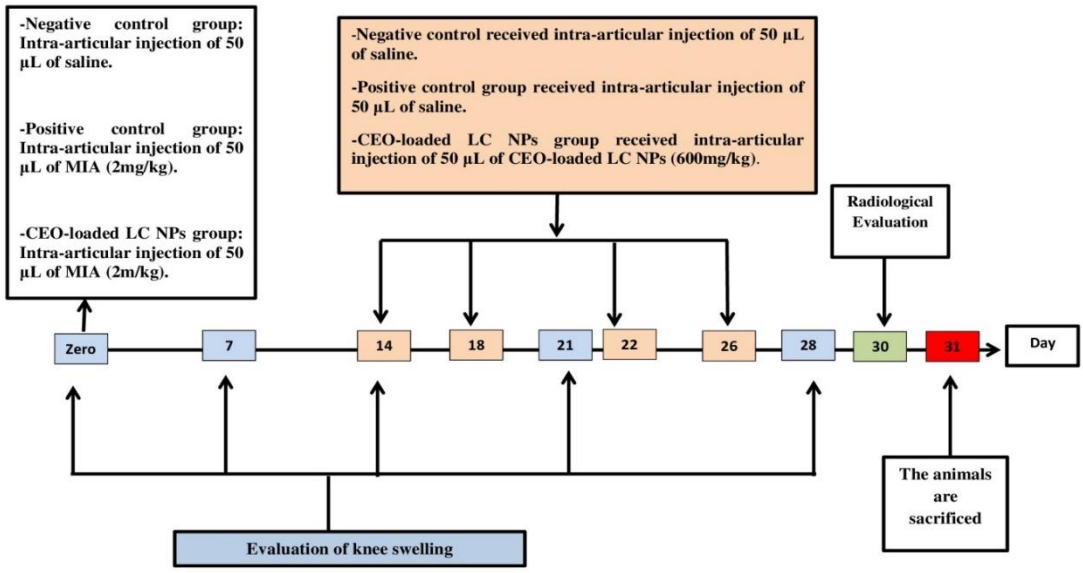

Figure 1: The experiment's timeline exhibits the animal groups, the induction of osteoarthritis (OA) on day zero, the initiation of therapy on days 14, 18, 22, and 26, and the animal sacrifice on day 31.

7. Knee Diameter Measurement (Swelling):

To evaluate the differences in the measurement of left knee joints across all animals, a manual Vernier caliper was utilized to measure the anterior-posterior circumference on days 0, 7, 14, 21, and 28 of the experiment following OA induction (18).

8. Radiographic Image Analysis:

On day 30 of the experiment, post-MIA injection, rats were anesthetized using a combination of ketamine and xylazine. For radiography, the entire body and both hind limbs were positioned on the detector plate 30 inches away from the X-ray emitter. The generator's X-ray dose was adjusted to kV (kVP =55) and mAs (3). A digital image was then downloaded.

9. Blood and serum collection:

After an 8–12 hour fast, blood samples (5 ml) were collected from the animals via cardiac puncture using sterile 5 ml syringes. Then the drawn blood was split into two tubes: one for the complete blood count test (K2-EDTA, No. 920202, AFCO, Jordan) and the other for serum separation (Gel and Clot Activator, AFCOVAC, Jordan). Employing the Abbott CELL-DYN Ruby hematology analyzer (USA), a full blood count was performed. After letting the blood coagulate for ten to fifteen minutes, the serum was separated using centrifugation at 3000 rpm for ten minutes. The serum was isolated and subjected to biochemical analysis.

10. Histopathological Analysis:

Histopathological investigations were done to detect any changes in the articulation bone after the rats were euthanized with ketamine and xylazine. All of the animals' left knee joints were collected and preserved in 10% neutral buffered formalin for 48 hours, then decalcified for two weeks with 20% Ethylenediaminetetraacetic acid (EDTA). The researchers excised the joints sagittally and cut sections with a paraffin thickness of

4-6 µm. They stained the sections with Hematoxylin and Eosin (H&E) before examining them under a light microscope (19).

11. ELISA Analysis:

Assessment of the inflammatory status performed by analyzing the serum pro-inflammatory mediators, including TNF-α (Cat. No. E0764 Ra), IL-6 (Cat. No. E0135 Ra), IL-10 (Cat. No. E0108Ra) ELISA kit (Bioassay technology laboratory, UK) and IL-1 beta (MAN0016903) utilized Thermo Fisher Scientific Rat ELISA kit. Lipid peroxidation and antioxidant activities evaluated by measuring serum MDA levels (Cat. No E0156Ra) and total antioxidant capacity (Cat. No E3901Ra), utilizing an ELISA kit (Bioassay technology laboratory, UK) in compliance with the manufacturer's instructions.

12. Statistics Analysis:

The data were expressed as mean ± standard deviation (S.D.) and statistically evaluated using one-way ANOVA analysis of variance followed by Tukey's tests using GraphPad Prism software (version 8). Unpaired T-tests were used to compare each group with the positive control group. The level of significance of difference was considered when p-value was < 0.01.

Results

1. Effect of CEO-LC NPs on Measurements of Knee Diameter:

After MIA injection, the knee diameter measurements were dramatically increased in both the positive and CEO-loaded LC NPs groups, in contrast with the measurements before injection. Nevertheless, the edema in the left knee joints was significantly reduced after two weeks of treatment with IAIs of CEO-LC NPs.

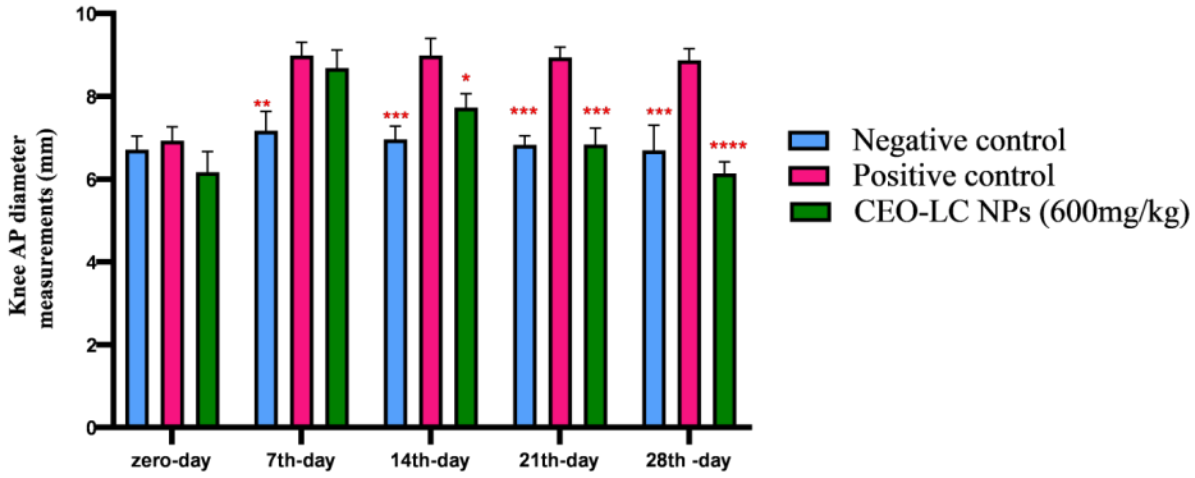

Figure 2: A summary of the anterior-posterior knee circumference measurements for the negative control, positive control, and CEO-LC NP treated groups at each time point. Significantly different in comparison with the positive control, the significant levels present as*(p < 0.05), ** (p < 0.01), * (p <0.001) and **** (p < 0.0001).**

2. Effect of CEO-LC NPs Using Radiographic Image:

Radiographic scanning was performed to declare modifications in the knee joints following CEO-LC NP therapy. The knee joints in the positive control group (Figure 3(b)) in comparison to those in the negative control group (Figure 3(a)) showed joint space narrowing and a distorted articular surface. In the meantime, the CEO-LC NP treatment substantially attenuated MIA-induced OA development and reduced joint deterioration, as shown by the slight degree of narrowing and the absence of visible sclerosis and osteophyte formation in CEO-loaded LC NP group. As described in Figure (3c).

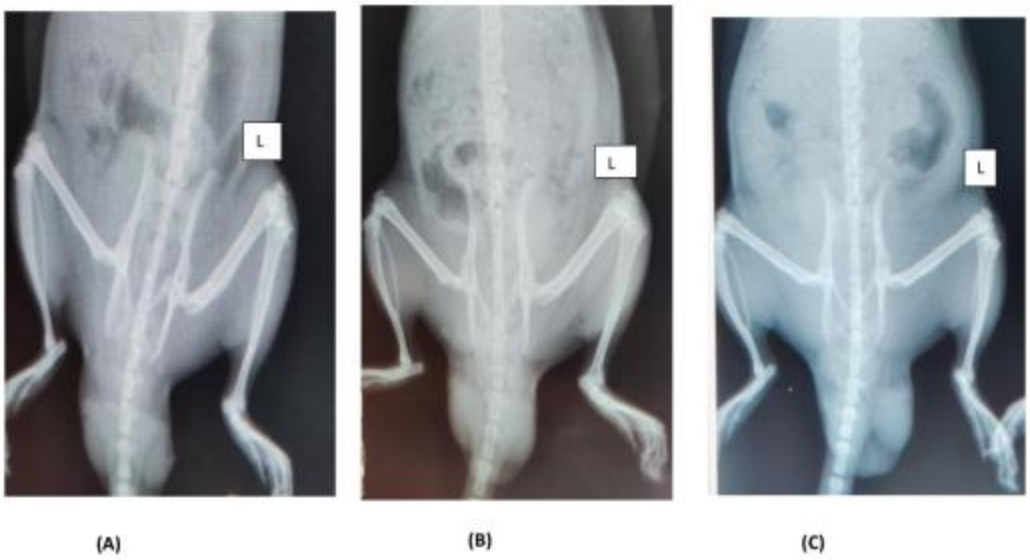

Figure 3: X-ray image demonstrate the left knee joints: (A) the negative control group, (B) positive control group illustrating changes seen on radiographs, such as osteophytes, joint space shortening, and erosion of the cartilage surface, and (C) CEO-LC NPs-treated group; with joints nearly resemble to those of the negative control group.

3. Effect of CEO-LC NPs on the Serum Levels of inflammatory mediators.

The positive control group revealed a significant augment in serum IL-6, TNF-α, C-reactive protein (C-RP), and interleukin-1β levels, while demonstrating a reduction in IL-10 levels compared to both the negative control and CEO-LC NPs groups. Meanwhile, the CEO-LC NPs-treated group exhibited marked reductions in IL-6 ($P = 0.0022$), TNF-α ($P = 0.0004$), C-RP ($P < 0.0001$), and IL-1β levels ($P < 0.0001$). However, there was an increase in IL-10 levels ($P = 0.0011$) compared to those of the positive and negative control groups. As depicted in Figure (4).

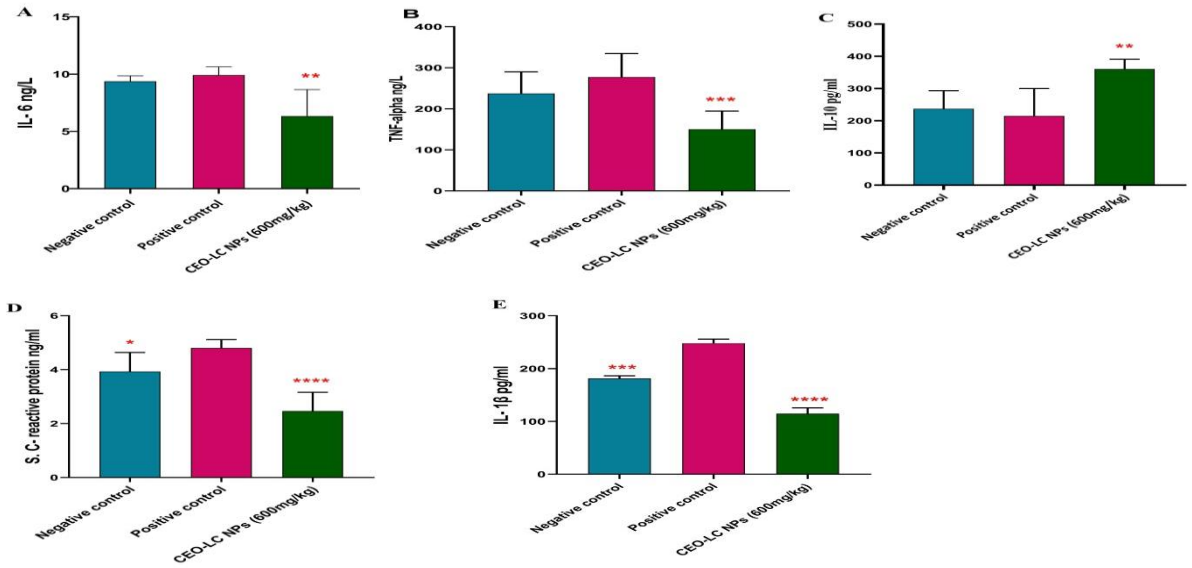

Figure 4: Effect of CEO-LC NPs on the serum levels of (A) interleukin-6, (B) tumor necrosis factor α, (C) interleukin 10, (D) C-reactive protein, and (E) interleukin-1 β as compared to positive control group; The values expressed as means ± SD and the significant levels present as * (P < 0.05), ** (P < 0.01), *(p < 0.001) and **** (p < 0.0001).**

4. Effect of CEO-LC NPs on malondialdehyde level and Antioxidant capacity.

In the positive control group, serum malondialdehyde levels (MDA) were significantly higher than those in the negative and CEO-LC NPs groups (P = 0.009, P = 0.0023), respectively. Meanwhile, the CEO-LC NPs group revealed a significant alleviation in MDA levels. The total antioxidant capacity levels in the CEO-LC NPs group were markedly increased in contrast to the positive and negative control groups (p = 0.0021, P = 0.0037), respectively. As illustrated in figure (5).

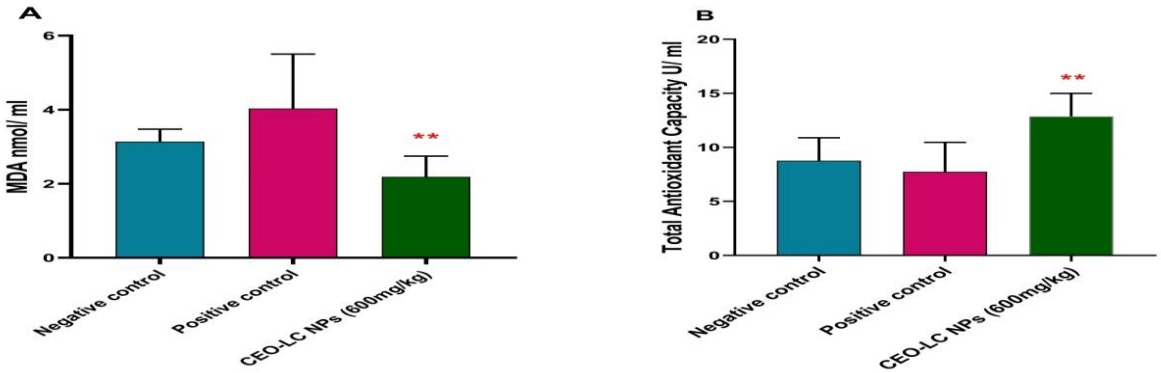

Figure 5: Effect of CEO-LC NPs on serum levels of (A) MDA and (B) total antioxidant capacity; The values expressed as means ± SD and the significant levels present as ** (P < 0.01), in contrast to positive group.

5. Effect of CEO-LC NPs on the hematological analysis:

Table 1 indicates a significant increase in total white blood cell count in the rat group with osteoarthritis compared to both the negative group and the group treated with CEO-LC. However, there were no differences in levels of red blood cells, hemoglobin, or mean corpuscular volume (MCV) among the groups. Conversely, platelet count, mean platelet volume (MPV), and platelet distribution width (PDW) levels were notably higher in the osteoarthritis group compared to both the negative group and the CEO-LC treated group.

Table 1. The summary of hematological parameters among the animal groups

Hematological parameter	Negative control group (Mean ± S.D)	Positive control group (Mean ± S.D)	CEO-LC (600mg/kg) treated group (Mean ± S.D)	P-value
White blood cell (WBC) (10^6/ µL)	8.85 ± 2.50	15.11 ± 1.51	9.20 ± 0.42	0.0001****
Lymphocyte (LYM)	6.02 ± 2.03	11 ± 1.36	6.9 ± 0.44	0.0001****
Monocyte	1.31 ± 0.38	1.60 ± 0.35	1.00 ± 0.35	0.022*
Granulocyte	1.5 ± 0.40	2.2 ± 0.97	1.48 ± 0.43	0.080
Red blood cell (RBC) (10^6/ µL)	7.91 ± 0.99	7.55 ± 0.63	8.05 ± 0.51	0.121
Hemoglobin (HGB) (g/dL)	14.6 ± 1.19	14.54 ± 0.73	15.26 ± 0.76	0.29
Mean corpuscular volume (MCV) fl	57.16 ± 1.42	58.76 ± 3.79	59.74 ± 3.66	0.32
Red blood cell distribution width (RDW)	17.7 ± 1.12	18.23 ± 2.28	16.97 ± 0.99	0.347
Platelet (PLT) (10^3/mm^3)	323.1 ± 55.95	440.1 ± 36.25	339.33 ± 30.03	0.0001***
Mean platelet volume (MPV)	6.55 ± 0.33	7.05 ± 0.59	6.12 ± 0.24	0.002**
platelet distribution width (PDW)	8.34 ± 0.80	9.41 ± 0.39	8.77 ± 0.36	0.007 **

The values expressed as means ± SD and the significant levels present as * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$), and **** ($P < 0.0001$) in contrast to positive group.

6. Effect of CEO-LC NPs on the Histopathological analysis:

Microscopic investigation in the negative control group revealed a normal histological architecture of the articulating bone figure (6 a), whereas in the positive control group, osteoarthritis was marked by regressive changes in bone and cartilage, including loss of cartilaginous matrix and Chondrocytes; in the other place, there was proliferation of chondrocytes forming clusters; in the bone, there was death of superficial osteocytes and increased osteoclastic activity causing microcyst formation (Figure 6 b). The CEO-LC NPs group received IAI of CEO-LC NP (600mg ⁄kg) on days 14, 18, 22, and 26; healing of degenerated tissue was detected. As demonstrated in Figure (6c).

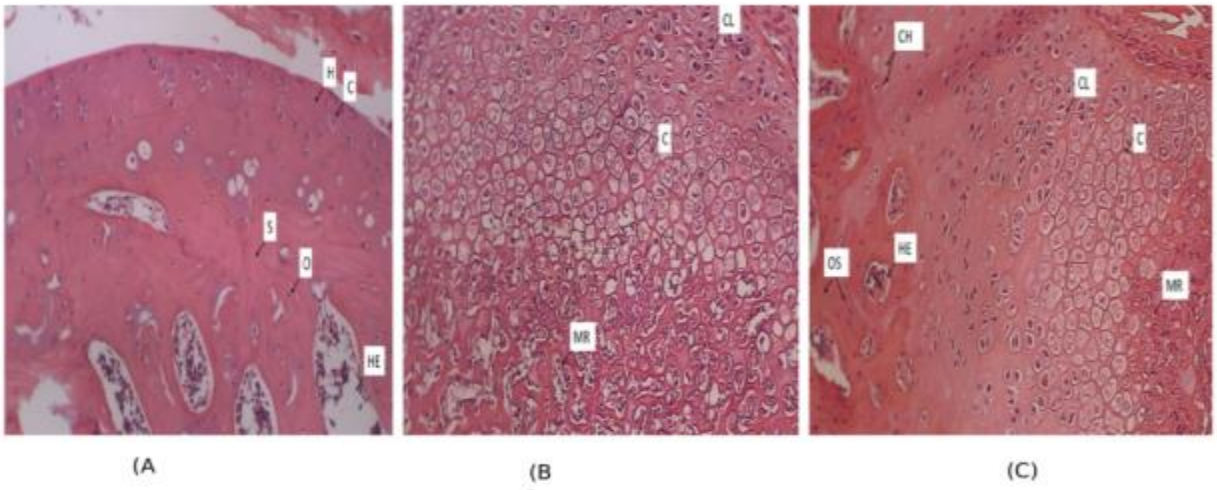

Figure 6: Photomicrographs of the left knee joints sections discoloration with hematoxylin and eosin-(H&E). Figure (6 A) negative control group, the head of articulating bone in knee joint revealed spongy bone, eosinophilic ground substance (s), with embedded osteocytes (o), and hematopoietic tissue (HE), covered by cartilage, hyaline eosinophilic ground substance (H) with scattered chondrocytes in their lacuna (C), there is no any pathological changes. Figure (6 B) positive control group, the regressive changes are marked in the articular cartilage, there is loss of cartilaginous matrix, and focal loss of Chondrocytes (C), in other place there was proliferation of chondrocytes forming clusters (CL), in bone there was death of superficial osteocytes and increased osteoclastic activity causing microcyst formation (MR). Figure (6 C) CEO-loaded LC NPs group in left side diseased area is seen, loss of cartilaginous matrix, focal loss of Chondrocytes (C), forming clusters (CL), in bone microcyst formation (MR), in right side pathological changes progressed to normal constitute of articulating bone indicated by covering cartilage, hyaline eosinophilic ground substance with scattered chondrocytes in their lacuna (CH), Under it regenerated bone tissue, eosinophilic ground substance with embedded osteocytes (OS).

Discussion

Research indicates that inflammation, oxidative stress, and excessive catabolic activity are associated with the development and progression of OA (20).

In the present study, we investigate the effectiveness of the CEO-LC NPs in suppressing oxidative stress and inflammatory mediators in a rat model of MIA-induced OA. CEO-LC NPs have demonstrated anti-inflammatory effects by decreasing levels of the pro-inflammatory mediators IL-6, IL-1β, TNF-α, and C-reactive protein. Moreover, they exhibit antioxidant properties by reducing oxidative status, as indicated by lower malondialdehyde levels, and by enhancing total antioxidant capacity.

An imbalance between pro- and anti-inflammatory mediators is a contributor to the pathophysiology of OA and results in low-grade inflammation and subsequent bone remodeling, synovial proliferation, and cartilage degradation (21). TNF-α and IL-6 are the most important inflammatory mediators in OA. They function as activators of numerous signaling pathways, which in turn activate more cytokines and pathological processes that increase COX-2 expression and, consequently, prostaglandin E2, which influences osteophyte production and cartilage deterioration (22).

Treatments with CEO-LC NPs have suppressed or inhibited the release of IL-1 β. The cytokine interleukin IL-1β is a key player in inflammatory reactions during infections

and immune-mediated illnesses. Moreover, in rheumatoid arthritis and osteoarthritis, IL-1 β promotes the creation and activity of matrix metalloproteinases and other enzymes that break down cartilage (23).

The changes noticed in hematological parameters related to inflammation in animal groups with osteoarthritis support the idea that inflammation plays a role in the development of osteoarthritis. Specifically, we found higher total white blood cell (WBC) levels in the osteoarthritis rat group, which is consistent with findings by Mehrani, Y. et al. (24). However, this contrasts with the study by Hira et al. (25), which reported no difference in total WBC levels in osteoarthritis patients, noting an increase only in septic arthritis.

In the recent study, significant differences were observed in mean platelet volume (MPV) values among rats with osteoarthritis. Previous research has shown that MPV is an inflammatory marker in various rheumatologic conditions, such as acute rheumatic fever, familial Mediterranean fever, rheumatoid arthritis, and ankylosing spondylitis (26, 27).

In the present experiment, histological and biochemical modifications were generated by MIA in the hyaline cartilage that were parallel to OA disease in humans (28). CEO-LC NPs were produced and analyzed for their effectiveness against oxidative stress and inflammatory mediators in a rat model.

Cardamom extract has demonstrated potent anti-inflammatory effects by impeding the release of pro-inflammatory cytokines, including IL-1b, TNF-a, and IL-8 (29). Cardamom extract inhibited the expression of COX-2 and alleviated the excrement of TNF-α, according to a study by Sreedharan, S. et al. (30). Souissi, M. et al. exhibited that cardamom extracts attenuated LPS-induced NF-kB signaling pathway activation (blocking of NF-kB transcription factor could be encouraging treatment and prophylaxis of chronic inflammatory disorders) (31).

The findings of this study revealed that treatment with IAI of CEO-LC NPs in the knee joint expressed anti-inflammatory activity by incrementing anti-inflammatory IL-10 release and reducing pro-inflammatory IL-6, TNF-a, C-RP, and IL-1 β expression. These results go along with those of previous studies. Nithia S. et al. (32) reported that CEO exhibits dose-dependent anti-inflammatory action by reducing the levels of pro-inflammatory cytokines TNF α, IL6, and IL1. Additionally, Cheshmeh S. et al. (33) observed significant reductions in serum levels of TNF-α, IL-6, and CRP. Furthermore, supplementation with green cardamom led to notable decreases in the gene expression of TNF-α and CRP.

In the ongoing study, MIA-induced OA considerably elevates MDA levels, signifying lipid peroxidation escalation. Furthermore, MIA-induced OA decreased antioxidant capacity. In comparison, treatment with IAI of CEO-loaded LC NPs in the knee joint amplified the activity of total antioxidant capacity and diminished oxidative stress marker levels (34, 35).

Ismail RF, et al. (35), found that the redox status exhibited significant positive impacts with CEO-LC NP treatment, as evidenced by increased levels of total antioxidant capacity (TAC), superoxide dismutase (SOD), and glutathione (GSH), along with decreased concentrations of malondialdehyde (MDA) and protein carbonyl (PC). Notably, the group receiving CEO-LC NP at 600 mg demonstrated substantially higher TAC levels and lower MDA levels compared to both the control group and other treatment groups.

Conclusion

The findings of the current study indicated that intra-articular administrations of CEO-LC nanoparticles enhance joint health in knee

osteoarthritis due to their anti-inflammatory and antioxidant characteristics. The anti-inflammatory mechanism of CEO-LC nanoparticles may involve the reduction of TNF-α, C-reactive protein, IL1, and IL6 levels, while total antioxidant capacity levels are increased. CEO-LC NPs are suggested to serve as a potentially efficient phytomedicine to prevent the progression of osteoarthritis and substitute for synthetic medications. However, additional research with extended treatment duration and larger sample sizes is needed to understand its underlying mechanisms for therapeutic applications.

Reference

1- Drummer D, McAdam J, Seay R, Ferrando A, Bridges Jr SL, Singh JA, et al. Osteoarthritis progression: mitigation and rehabilitation strategies. Frontiers in Rehabilitation Sciences. 2021;2:724052.
2- Choi DJ, Choi S-I, Choi B-R, Lee Y-S, Lee DY, Kim GS. Cartilage protective and anti-analgesic effects of ALM16 on monosodium iodoacetate induced osteoarthritis in rats. BMC complementary and alternative medicine. 2019;19(1):1-15.
3- Chern C-M, Zhou H, Wang Y-H, Chang C-L, Chiou W-F, Chang W-T, et al. Osthole ameliorates cartilage degradation by downregulation of NF-κB and HIF-2α pathways in an osteoarthritis murine model. European journal of pharmacology. 2020;867:172799.
4- Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. Nature Reviews Cancer. 2013;13(11):759-71.
5- Subramoniam A, Madhavachandran V, Gangaprasad A. Medicinal plants in the treatment of arthritis. Ann Phytomed. 2013;2(1):3-36.
6- Sánchez-Ramos M, Alvarez L, Romero-Estrada A, Bernabé-Antonio A, Marquina-Bahena S, Cruz-Sosa F. Establishment of a cell suspension culture of Ageratina pichinchensis (Kunth) for the improved production of anti-inflammatory compounds. Plants. 2020;9(10):1398.
7- Morsy NF. A short extraction time of high quality hydrodistilled cardamom (Elettaria cardamomum L. Maton) essential oil using ultrasound as a pretreatment. Industrial Crops and Products. 2015;65:287-92.
8- Joshi R, Sharma P, Sharma V, Prasad R, Sud R, Gulati A. Analysis of the essential oil of large cardamom (Amomum subulatum Roxb.) growing in different agro-climatic zones of Himachal Pradesh, India. Journal of the Science of Food and Agriculture. 2013;93(6):1303-9.
9- Mazumder J, Kumria R, Pathak D. Evaluation of synergistic antimicrobial activity and antioxidant activity of blend of essential oil contains fennel, coriander, ajowan and caraway. IOSR J Pharm Biol Sci. 2014;9(1):87-94.
10- Soares S, Sousa J, Pais A, Vitorino C. Nanomedicine: principles, properties, and regulatory issues. Frontiers in chemistry. 2018;6:360.
11- Kumar S, Randhawa JK. High melting lipid based approach for drug delivery: Solid lipid nanoparticles. Materials Science and Engineering: C. 2013;33(4):1842-52.
12- Beloqui A, Solinís MÁ, Rodríguez-Gascón A, Almeida AJ, Préat V. Nanostructured lipid carriers: Promising drug delivery systems for future clinics. Nanomedicine: Nanotechnology, biology and medicine. 2016;12(1):143-61.
13- Gaba B, Fazil M, Ali A, Baboota S, Sahni JK, Ali J. Nanostructured lipid

(NLCs) carriers as a bioavailability enhancement tool for oral administration. Drug delivery. 2015;22(6):691-700.
14- Pathak K, Keshri L, Shah M. Lipid nanocarriers: influence of lipids on product development and pharmacokinetics. Critical Reviews™ in Therapeutic Drug Carrier Systems. 2011;28(4).
15- Nahr FK, Ghanbarzadeh B, Hamishehkar H, Kafil HS. Food grade nanostructured lipid carrier for cardamom essential oil: Preparation, characterization and antimicrobial activity. Journal of functional foods. 2018;40:1-8.
16- Takahashi I, Matsuzaki T, Kuroki H, Hoso M. Induction of osteoarthritis by injecting monosodium iodoacetate into the patellofemoral joint of an experimental rat model. PLoS One. 2018;13(4):e0196625.
17- Fatemeh Y, Siassi F, Rahimi A, Koohdani F, Doostan F, Qorbani M, et al. The effect of cardamom supplementation on serum lipids, glycemic indices and blood pressure in overweight and obese pre-diabetic women: a randomized controlled trial. Journal of Diabetes & Metabolic Disorders. 2017;16:1-9.
18- Badawi AA, El-Laithy HM, Nesseem DI, El-Husseney SS. Pharmaceutical and medical aspects of hyaluronic acid–ketorolac combination therapy in osteoarthritis treatment: radiographic imaging and bone mineral density. Journal of Drug Targeting. 2013;21(6):551-63.
19- Hamdalla HM, Ahmed RR, Galaly SR, Naguib IA, Alghamdi BS, Ahmed OM, et al. Ameliorative Effect of Curcumin Nanoparticles against Monosodium Iodoacetate-Induced Knee Osteoarthritis in Rats. Mediators of Inflammation. 2022;2022(1):8353472.
20- Ansari MY, Ahmad N, Haqqi TM. Oxidative stress and inflammation in osteoarthritis pathogenesis: Role of polyphenols. Biomedicine & pharmacotherapy. 2020;129:110452.
21- Robinson WH, Lepus CM, Wang Q, Raghu H, Mao R, Lindstrom TM, et al. Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis. Nature Reviews Rheumatology. 2016;12(10):580-92.
22- Primorac D, Molnar V, Matišić V, Hudetz D, Jeleč Ž, Rod E, et al. Comprehensive review of knee osteoarthritis pharmacological treatment and the latest professional societies' guidelines. Pharmaceuticals. 2021;14(3):205.
23- Elguindy NM, Yacout GA, El Azab EF, Maghraby HK. Chemoprotective effect of Elettaria cardamomum against chemically induced hepatocellular carcinoma in rats by inhibiting NF-κB, oxidative stress, and activity of ornithine decarboxylase. South African journal of botany. 2016;105:251-8.
24- Mehrani Y, Rahimi Junqani R, Morovati S, Mehrani H, Karimi N, Ghasemi S. The Importance of Neutrophils in Osteoarthritis: Current Concepts and Therapeutic Perspectives. Immuno. 2023;3(3):250-72.
25- Hira[1] S, Tamam C. Diagnostic value of hematological parameters in patients with osteoarthritis. Cukurova Medical Journal. 2017;42(1):120-5.
26- Özer S, Yılmaz R, Sönmezgöz E, Karaaslan E, Taşkın S, Bütün İ, et al. Simple markers for subclinical inflammation in patients with Familial Mediterranean Fever. Medical Science Monitor: İnternational medical journal of experimental and clinical research. 2015;21:298.

27- Sert A, Aypar E, Odabas D. Mean platelet volume in acute rheumatic fever. Platelets. 2013;24(5):378-82.
28- Naveen SV, Ahmad RE, Hui WJ, Suhaeb AM, Murali MR, Shanmugam R, et al. Histology, glycosaminoglycan level and cartilage stiffness in monoiodoacetate-induced osteoarthritis: comparative analysis with anterior cruciate ligament transection in rat model and human osteoarthritis. International Journal of Medical Sciences. 2014;11(1):97.
29- Jiang J, Cai M. Cardamonin inhibited IL-1β induced injury by inhibition of NLRP3 inflammasome via activating nrf2/NQO-1 signaling pathway in chondrocyte. Journal of Microbiology and Biotechnology. 2021;31(6):794.
30- Sreedharan S, Nair V, Cisneros-Zevallos L. Protective Role of Phenolic Compounds from Whole Cardamom (Elettaria cardamomum (L.) Maton) against LPS-Induced Inflammation in Colon and Macrophage Cells. Nutrients. 2023;15(13):2965.
31- Souissi M, Azelmat J, Chaieb K, Grenier D. Antibacterial and anti-inflammatory activities of cardamom (Elettaria cardamomum) extracts: Potential therapeutic benefits for periodontal infections. Anaerobe. 2020;61:102089.
32- Nithya S. Anti-inflammatory effect of Elettaria cardamom oil on carrageenan-induced paw edema using rats based on tumor necrosis factor α, interleukin 6, and interleukin 1 levels in serum. Asian J Pharm Clin Res. 2018;11:207.
33- Cheshmeh S, Ghayyem M, Khamooshi F, Heidarzadeh-Esfahani N, Rahmani N, Hojati N, et al. Green cardamom plus low-calorie diet can decrease the expression of inflammatory genes among obese women with polycystic ovary syndrome: a double-blind randomized clinical trial. Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity. 2022;27(2):821-30.
34- Alkhalifah EA, Alobaid AA, Almajed MA, Alomair MK, Alabduladheem LS, Al-Subaie SF, et al. Cardamom Extract Alleviates the Oxidative Stress, Inflammation and Apoptosis Induced during Acetaminophen-Induced Hepatic Toxicity via Modulating Nrf2/HO-1/NQO-1 Pathway. Current Issues in Molecular Biology. 2022;44(11):5390-404.
35- Ismail RF, Hassan MA, Moustafa M, Al-Shehri M, Alazragi RS, Khojah H, et al. The Influence of a Nanoemulsion of Cardamom Essential Oil on the Growth Performance, Feed Utilization, Carcass Characteristics, and Health Status of Growing Rabbits under a High Ambient Temperature. Animals. 2023;13(18):2990.