OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

Pharmacological Screening of Anti-arthritic Effect of Biological Synthesis ZnONPs by *Syzygium aromaticum* in Rats

Rana Mahmood Ahmad*, Orooba M.S. Ibrahim

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Abstract | The study was conducted to synthesize zinc oxide nanoparticles (ZnONPs) biologically by using *Syzagium aromaticam* extract and evaluate the effect against Polyarthritis produced by complete Freund's Adjuvants injected (S.c.) into the hind paw. The confirmation of the synthesis and characterization of ZnONPs was achieved through the utilization of a UV-spectrophotometer and SEM. For this purpose, eighteen adult male rats 200 ± 50 g at six months of age will be randomly divided into three groups, each containing six rats. Groups 2 and 3 were treated daily for 14 days from the day of Freund's adjuvant injection with SaZnONPs and piroxicam at doses 2.5 and 5 mg/kg B.W., respectively, while (Group 1) was positive control. Blood samples were obtained using cardiac puncture at three time points during the experiment: at the beginning (zero time) and on the 12th and 21st day. These samples were collected specifically for serum collection. In the present investigation, the administration of SaZnONPs to infected rats yielded noteworthy reductions in the levels of the pro-inflammatory cytokine TNF-α. SaZnONPs demonstrated an elevation in the levels of the anti-inflammatory cytokine IL-10, which is known to play a crucial role in safeguarding tissues against damage caused by inflammation. These results led us to hypothesize that SaZnONPs therapy speeds up the switch from an inflammatory to an anti-inflammatory response during the healing process. The histopathological observation showed that a section of a joint in groups treated with SaZnONPs had a normal joint cavity with normal articular cartilage compared with other treated groups. Accordingly, the findings of the present study suggested that ZnONPs reinforced with clove can be anti-arthritis treatments and may be utilized as a substitute for medicines currently on the market.

Keywords | Zinc oxide nanoparticles; anti-inflammatory; Rheumatoid arthritis; Green synthesis, Rats

Received | August 27, 2023; **Accepted** | September 20, 2023; **Published** | December 01, 2023
***Correspondence** | Rana Mahmood Ahmad, Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq; **Email:** auroba.m@covm.uobaghdad.edu.iq
Citation | Ahmad RM, Ibrahim OMS (2023). Pharmacological screening of anti-arthritic effect of biological synthesis znonps by *Syzygium aromaticum* in rats. Adv. Anim. Vet. Sci. 11(12): 2053-2059.
DOI | http://dx.doi.org/10.17582/journal.aavs/2023/11.12.2053.2059
ISSN (Online) | 2307-8316

INTRODUCTION

Arthritis is a group of severe inflammatory disorders characterized by a mild to severe degree of inflammation and degradation in the joints and auxiliary components such as bones and cartilages. Inflammation manifests itself in classic arthritic symptoms such as swelling and stiffness in a single or numerous joints, resulting in pain and suffering for long periods of time. Rheumatoid arthritis, osteoarthritis, infectious arthritis, psoriatic arthritis, lupus arthritis, fibromyalgia, ankylosing spondylitis, gout, and juvenile arthritis are some of the extant types of arthritis (Hasan et al., 2014; Singha et al., 2020). The most common arthritis-related conditions are osteoarthritis (OA), rheumatoid arthritis (RA), and gout. However, the major focus of this research will be on the first two cat-

egories, OA and RA. Both of these conditions have similar pathological manifestations, but RA is an autoimmune condition while OA gradually worsens with age (Bandyopadhyay et al., 2018). The cause of RA is unknown, but there is evidence linking the autoimmune response to joint inflammation, with genetics and some environmental factors like smoking and infectious diseases playing a major role as contributing factors (Faiq et al., 2019). Contrarily, OA causes inflammation in nearly every joint component, including the cartilage, bones, and ligaments, which leads to stiffness and, ultimately, pain in the flexing joints. Both of these arthritic disorders are chronic because they last a long time and have not yet been permanently cured, which the most is depressing aspect supported by literature (Singha et al., 2020). Non-steroidal anti-inflammatory medicines (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs), glucocorticoids, and certain biological therapies have so far been used to treat these debilitating illnesses (Halasz and Carpenter, 2022). However, extended use of these drugs has a number of drawbacks, including hepatotoxicity, renal toxicity, gastrointestinal hemorrhage, immunosuppression, hyperglycemia, and so on (Naser et al., 2011; Mahdi et al., 2012). Therefore, the search for innovative pharmaceutical agents has emerged as a practical alternative to conventional anti-inflammatory drugs. As a result, many compounds have been synthesized to selectively target cytokines involved in the inflammatory response and disease progression.

Nanotechnology is a novel and intriguing science that aims to create unique materials at the nanoscale scale. As a type of nanomaterial, these nanoparticles have prospective uses in the areas of anti-bacterial, anti-fungal, anti-inflammatory, wound healing, and optical characteristics (Hasan and Raheem, 2021). ZnO NPs are widely employed as a form of nanomaterial in medical devices , textile industry, cosmetics, and other industries (Husain et al., 2019). Because of their high specific surface area, biocompatibility, UV light absorption, scattering capabilities, and antibacterial properties (Shaba et al., 2021). Physical and chemical processes are used to synthesize ZnO NPs. However, this approach has several limitations, including elevated energy consumption, low purity, inconsistent particle size distribution, increased expenses, substantial secondary waste generation, and irreversible environmental harm (Salih et al., 2017; Xu et al., 2021). The increasing demand for ZnO NPs has raised concerns over their synthesis through environmentally sustainable approaches. This concern stems from the widespread incorporation of environmental protection principles into societal expectations. These are environmentally benign and biological synthesis techniques that use microbial enzymes, fungus, plants, or plant extracts (Al-Ghareebawi et al., 2021; Ayad et al., 2019). This biological nanoparticle synthesis is known as green nanoparticle synthesis (Husain et al., 2019).

Clove (Syzygium aromaticum) is a tree with median size (8-12m) belong to myrtaceae family in the east of Indonesia, it is also cultured in india, Malaysia, and Sri Lanka (Farhan et al., 2023). Clove is composed of numerous types and groups of chemical components like phenolic and hydrocarbon components, monoterpenes, the main compounds in clove oil are eugenol followed by eugenyl acetate and β-caryophyllene, eugenol is a phenylpropene compound, consider as the principle bioactive ingredient of clove essential oil gained from Eugenia Caryophyllata buds and leaves (Katariya et al., 2023). Clove oil is used commercially for various medicinal treatment and in the industry of fragrance, is consider as one of the spices which can be possibly used as additives in the nutrients, particularly in meat processing, to replace chemical preservatives as a result of their antioxidant, antimicrobial and ant inflammatory properties (Elbestawy et al., 2023; Ali and Ibrahim, 2023). ZnO NPs manufactured from plant extracts have many applications where they can be used as antibacterial and anti-inflammatory agents (Xu et al., 2021).

The rationale for this study is the inadequacy of previous research to evaluate the properties of clove-enhanced ZnONPs. Hence the primary objective of the present study was to evaluate the anti-arthritic properties of ZnONPs combined with clove in a rat model of arthritis produced by complete Freund's adjuvant (CFA).

MATERIAL AND METHODS

Collection of Plant

Clove (Syzygium atomaticum) Buds were gathered from the Baghdad local market and disinfected using sodium hypochlorite solution (5% NaOCl), followed by three washes with distilled water and preservation for drying. The plant preparation was done in the college of Veterinary Medicine / Department of Physiology, Biochemistry and Pharmacology.

Preparation of clove oil

Flower buds (10 g) were washed (2–3) times in deionized water, allowed to dry out in the sun for 144 hours, and then powdered using a clean electric blender and stored in sterile polyethylene test sacks before to use. The obtained powder was macerated with Petroleum ether in a glass screw-top reagent vial at a 1:6 (w/v) ratio for 96 hours. As a result, the supernatant was filtered through two layers of muslin fabric before being centrifuged at 4000 rpm for 10 minutes to recover the supernatant. The supernatant was sieved using Whatman No. 1 filter paper, and the filtrate that resulted was regarded as mother extract and stored aseptically at 4

Co for later use (Ishaq et al., 2019).

Biosynthesis And Characterization Of Zno Nanoparticles

Clove oil extract and a solution of zinc nitrate crystal [$Zn(NO_3)_2 \cdot 6H_2O$] with a concentration of 0.1M have been employed in the synthesis of ZnONPs (Anvarinezhad et al., 2020; Jayachandran et al., 2021). The physical properties of biologically synthesized ZnONPs were investigated using a UV-visible spectrophotometer operating within the wavelength range of 200-800 nm (Lopez-Miranda et al., 2023), and SEM is used to determine their size, shape, and surface morphology (Hussain et al., 2019; Ayad et al., 2019).

Evaluation Of Anti-Inflammatory Properties By Rheumatoid Arthritis Induction

Test Animal: Eighteen adult male rats 200 ± 50 g at 6 months of age. Rats were obtained from the Veterinary Medicine College's animal house for Baghdad University. They were kept in groups of six per cage for two weeks to acclimate. The room temperature was kept between (21 and 25 degrees Celsius), and the air in the room was changed on a constant basis utilizing a ventilation vacuum and a light/dark cycle of 12:12 hours each day. Every two days, the litter in the cages was replaced. The animals were fed freshly prepared ration pellets.

Rheumatoid Arthritis Induction: The experimental procedure involved the induction of rheumatoid arthritis in rats with the subcutaneous injection of 0.1ml of complete Freund's adjuvant into the planter region of the right hind paw (Ismail et al., 2022; El-Tanbouly et al., 2022).

Experimental Design: A total of eighteen rats were randomly assigned to three groups, with each group consisting of six rats. Group 1 was the positive control: that animals were infected and leave without treatment. On the other hand, Group 2 received a daily oral administration of SaZnONPs (2.5 mg/kg body weight) for 14 days, starting from the day of Freund's adjuvant injection. Group 3 received Piroxicam orally at a dosage of (5mg/kg) of body weight daily for 14 days, starting from the day of Freund's adjuvant injection.

Specimen Preparation: The animals were administered anesthesia with intramuscular injection of Ketamine (90 mg/kg) and Xylazine (40 mg/kg) (Hussain et al., 2019). Blood samples were obtained from the subjects on days 0 and 21 of the trial using the cardiac puncture technique. The serum was obtained from a coagulated blood sample using centrifugation at 2500 revolutions per minute for 15 minutes. Subsequently, the serum was preserved by freezing it at -20 degrees Celsius until it was ready for use. The assessment of TNF-α and IL-10 was conducted using an ELISA kit according to the instructions provided by the kit manufacturer. The histological changes of joint tissue were examined after the euthanization of animals.

Statistical Analysis

The data were evaluated through statistical analysis using Microsoft's SAS software (Statistical Analysis System - version 9.1). The data underwent statistical analysis with the application of Two Way Analysis of Variance (ANOVA) at a significance level of ($P<0.05$), employing least significant differences (LSD). A post hoc test was conducted to evaluate the presence of a significant difference among the means (SAS, 2012).

RESULTS

Biosynthesis Of Zinc Oxide Nanoparticles By *S.Arumaticum* Extract

The current study demonstrated that Clove (*S. aromaticum*) oil extract was an excellent source of stable ZnO NPs. The reduction of zinc nitrate to zinc oxide after exposure to *S. aromaticum* extract was followed by a color change of the solution to light yellow within 24 hours, according to the results of these studies. The maximum absorbance of zinc oxide nanoparticles is demonstrated to peak at a surface Plasmon resonance at 329.62 nm by UV-Vis spectral analysis. SEM provided additional information into the size and form features of the synthesized ZnONPs. The results showed that the particles were crystalline in form, with the majority of the nanoparticles being hexagonal in shape and ranging in size from 32 to 59 nm (Figure 1).

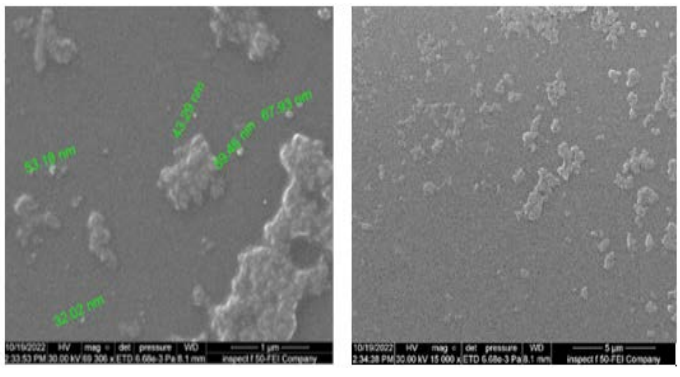

Figure 1: SEM image of ZnO NPs synthesized by clove *(S. aromaticum)* oil extract

Effect Of Saznonps As Anti-Arthritis -Inflammatory Marker

Measurement of Tnf-A Levels: The serum TNF- α levels in the different groups at day 21 in rat injection with Freund's Adjuvant are shown in (Table 1), we found that TNF-α not statistically significant ($P> 0.05$) in the

SaZnONPS treated group compared with the level that before injection while piroxicam and positive control groups record elevated significantly (P≤0.05) in TNF-α concentrations when compared with level that before infection.

Table 1: Effect of Sa ZnONPs and clove oil extract on TNF-α level after 21 days on Adjuvant-induced arthritis

Groups	TNF-α (Pg/ml)	
	Before induction	After 21 days
(G1) Positive Control	332.50±3.39 Aa	565.30±7.34 Ab
(G2) Treat with SaZnNPs 2.5 mg/Kg.BW	335.43 ±6.38 Aa	336.20 ±4.59 Ca
(G3) Treat with piroxicam 5 mg/kg.BW	336.51 ± 8.30 Aa	348.50 ±5.99 Bb
LSD value	4.1*	

N= 6, Means having with the different capital letters in same column and small letters in same row differed significantly, * (P≤0.05)

Table 2: Effect of Sa ZnONPs and clove oil extract on IL-10 level after nine days on Adjuvant-induced arthritis

Groups	IL- 10 (Pg/ml)	
	Before induction	After 21 days
(G1) Positive Control	131.04 ±3.25 Ab	47.83±6.57 Ca
(G2) Treat with SaZnNPs 2.5 mg/Kg.BW	130.22±4.22 Aa	128.32±7.44 Aa
(G3) Treat with piroxicam 5 mg/kg.BW	132.92±5.41 Ab	110.55±5.62 Ba
LSD value	5.20*	

N= 6, Means having with the different capital letters in same column and small letters in same row differed significantly, * (P≤0.05).

Measurement of Il-10 Levels: Serum interleukin-10 values were measured in pg/ml for all groups and are shown in (Table 2). Before induction, there were no significant differences (P > 0.05) between any of the groups; however, at day 21 after treatment with SaZnONPs, serum IL-10 concentrations significantly (P> 0.05) increased in comparison to the other treated groups and significantly returned to normal; additionally, groups treated with piroxicam showed increase level of Il-10 after 21 day but still less than SaZnONPs. Positive control groups that showed no any improvement in the anti-inflammatory cytokines level (IL-10) in serum.

- Microscopically Examination Of Joint Tissue

DISCUSSION

This study aims to investigate the potential positive impacts associated with the use of ZnONPs on the progression rates of rheumatoid arthritis (RA). A recent study has focused on the production of nanoparticles utilizing an ecologically friendly approach.

Green sources are used as a stabilizing and reducing agent in the synthesis of shape- and size-controlled nanoparticles (Salih et al., 2016). With the aid of *S. arumaticum* extract, ZnONPs were created. A wide peak in the UV-Vis spectrum was visible at 329.26 nm because to ZnO NP's surface Plasmon absorption, that result agree with (Ali et al., 2016), who concur with our findings.The SEM analysis is utilized to describe the size, shape, morphology, and dispersion of the created ZnONPs. The resulting ZnONPs feature multiple aggregated particles and an irregular spherical form, as shown in the SEM micrographs. With a diameter ranging from 32 to 59 nm, that finding agrees with (Hussain et al., 2019).

Adjuvant-induced arthritis in rats has been authorized as an experimental model for studying rheumatoid arthritis (RA) etiology (Qin et al., 2023; Al-Shammari et al., 2023). Complete CFA produced arthritis in male Wistar albino rats because higher amounts of inflammatory cytokines were found in the serum of rats given CFA. Autoimmunity, persistent infammation, and joint degradation are the three phases of rheumatoid arthritis (Omran et al., 2022). Zinc supplements have been shown to benefit the elderly population by reducing inflammation and oxidative stress (Hosseini et al., 2021). In the current work, SaZnO NPs decreased adjuvant-induced elevated TNF- levels in rats. Tumour necrosis factor alpha (TNF-α) is a prominent inflammatory cytokine that plays a crucial role in the development of rheumatoid arthritis (RA) (Parlindungan et al., 2023). Its involvement in bone resorption and cartilage remodeling is mediated by the maturation of osteoclast cells and the inhibition of collagen synthesis. Moreover, they enhance other inflammatory processes, including angiogenesis, fibrosis, and cell adhesion. The efficacy of SaZnONPs in mitigating inflammation has been well-documented through various mechanisms. The anti-inflammatory properties of these substances have been ascribed to their capacity to inhibit the synthesis of nitric oxide, NF-κB, and caspase-1, which is responsible for the activation of pro-IL-1β. Consequently, they can suppress the production of IL-1β and TNF-α (Jiang et al., 2018; Agarwal et al., 2019).

Interleukin 10 (IL-10) is an immunoregulatory cytokine produced by Treg cells that is important in the prevention of tissue damage caused by inflammation (Li et al., 2023).

The production of this substance is predominantly attributed to monocytes/macrophages, and it exerts a significant anti-inflammatory influence on the activities of T-lymphocytes and monocytes. This is achieved by the suppression of T-lymphocyte proliferation and the reduction of antigen presentation by monocytes. In the present investigation, administering SaZnONPs to rats with infections led to a notable elevation in the levels of the anti-inflammatory cytokine IL-10 while concurrently causing a reduction in the levels of the pro-inflammatory cytokine TNF-α. These results led us to hypothesize that SaZnONPs therapy speeds up the switch from an inflammatory to an anti-inflammatory response during the healing process (Mukherjee, 2023).

Our study were registered the histopathological section of knee joint in healthy rat showed normal appearances for the joint cavity, articular surface, articular hyaline cartilage and epiphyseal bone plate (Figure 2). While the histopathological examination after 21 days of infection with RA was showed sever arthritis which characterized by occupied joint cavity with necrotic tissue debris with marked degeneration and necrosis of articular cartilage. The joint capsule revealed marked thickening of articular capsule that associated with sever vascular congestion and infiltration of mononuclear leukocytes mainly lymphocytes and necrosis of collagen fibers (Figure B). the histopathological observations of the knee joint in the groups treated with SaZnONPs showed the normal appearance of the joint cavity with the normal articular cartilage and normal inner synovial membrane, while the outer fibrous capsule revealed mild thickening associated with fibroplasia, infiltration of mononuclear leukocytes and angiogenesis (figure C) as compared to other groups of study that treated with piroxicam that showed moderate arthritis that characterized by normal joint cavity, marked degeneration and necrosis of once of articular cartilage, slightly thickening of articular capsule and degeneration of inner fibrous membrane of joint capsule (Figure D).This result is may be due to the synergistic activity of SaZnoNPs in reduced joint inflammation, synovial immune cell infiltration and synovial hyperplasia (Singha et al., 2023). SaZnONPs in promoting the proliferation, differentiation and maturation of keratinocytes through the stimulation of stem cells. These keratinocytes contain higher levels of Vascular Endothelial Growth Factor (VEGF) (Choi et al., 2021). VEGF plays a crucial role in facilitating the development of new blood vessels (Cadau et al., 2022). VEGF It has been shown to play a role in improves blood circulation, ensuring that more nutrients and oxygen are reached to infected joint in groups that treated with SaZnONPs.The proliferation, differentiation and maturation of keratinocytes by SaZnONPs increased the rate of inflammatory healing significantly with respect to other groups. On the other hand, SaZnONPs could drive the collagen synthesis, sclerotic bone or osteophytes and proliferative cartilage thereby promoting joint healing (Khan et al., 2023). The microscopic results showed also that SaZnONPs enhanced early re-epithelialization which appeared at day 21 in infected group.

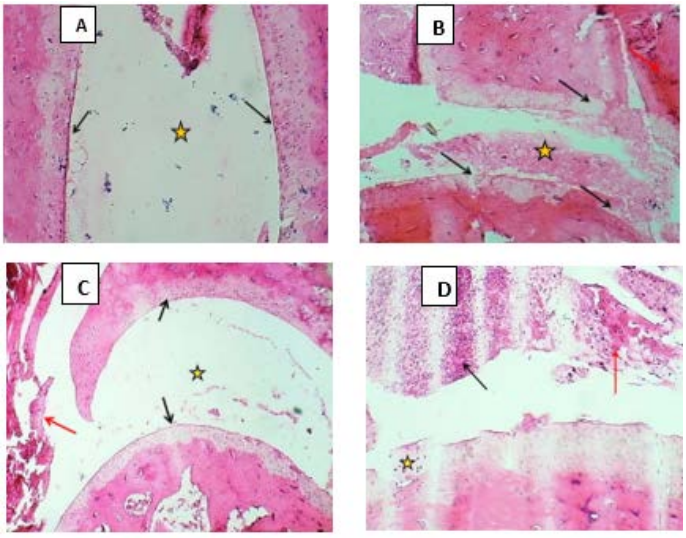

Figure 2: Microscopically examination of joint tissue in A-control negative: section of joint shows normal joint cavity (Asterisk) & normal articular surface (Black arrows). B- (G1) section of joint shows filled of joint cavity with necrotic tissue debris (Asterisk), degeneration of articular cartilage (Black arrows) & thickening of articular capsule (red arrow).C- (G2) section of joint that shows normal joint cavity with normal articular cartilage (Black arrows), normal outer fibrous joint capsule with normal inner synovial membrane (Red arrow). D- (G3) section of joint that shows moderate arthritis characterized by degeneration with necrosis of once of articular cartilage with marked infiltration of MNCs (Black arrow), irregular articular surface (Asterisk). H&E stain. 100x

CONCLUSION AND RECOMMENDATION

This study introduced the synthesis of ZnONPs through clove oil extract, a widely recognized botanical source renowned for its therapeutic properties. This approach is characterized by its environmentally friendly nature, lack of negative impact, and overall simplicity compared to alternative methods. "Activity" refers to a broad range of actions or behaviors individuals engage in. The therapeutic application of SaZnONPs has been observed to expedite the transition from an inflammatory to an anti-inflammatory response during the healing process. This effect is achieved through inhibiting pro-inflammatory cytokines, specifically TNF-α, which are pivotal in the inflammatory pathway associated with the development of rheumatoid

arthritis (RA).

Additionally, SaZnONPs have been found to elevate the anti-inflammatory cytokine IL-10 levels, which play a crucial role in safeguarding tissues against damage caused by inflammation. According to the data, the present study's findings indicate that ZnONPs, when combined with clove, have promising properties as an anti-arthritis agent. Consequently, these nanoparticles have the potential to serve as a viable substitute for existing commercially accessible treatments.

ACKNOWLEDMENTS

The authors would like to thank the administrators of College of Veterinary Medicine, University of Baghdad for providing all facilities during the experimental procedures.

NOVELTY STATEMENT

The novelty of the study is focus on physiologically active for green syntheses ZnONPs that can be employed as novel anti-inflammatory pharmaceuticals due to the great side effect of present drugs and some case of chronic inflammation that need long time use medicines, it is imperative to seek into alternate sources of anti-inflammatory medications.

CONFLICTOF INTERESTS

There are no competing interests.

AUTHORS CONTRIBUTION

These authors each contributed equally

REFERENCES

Agarwal H, Nakara A, Shanmugam V K (2019). Anti-inflammatory mechanism of various metal and metal oxide nanoparticles synthesized using plant extracts: A review. Biomed. Pharmacother., 109: 2561-2572. https://doi.org/10.1016/j.biopha.2018.11.116.

Al-Ghareebawi A M, Al-Okaily B N, Ibrahim O M S (2021). Characterization of Zinc Oxide Nanoparticles Synthesized by Olea Europaea Leaves Extract (Part L). Iraqi J. Agricult. Sci., 52(3): 580-588.

Ali K, Dwivedi S, Azam A, Saquib Q, Al-Said M S, Alkhedhairy A A, Musarrat J (2016). Aloe vera extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates. J. Colloid Inter. Sci., 472: 145-156. http://dx.doi.org/10.1016/j.jcis.2016.03.021.

Ali B M, Ibrahim O (2023). Antifungal Activity of Clove (*Syzygium aromaticum*) Essential Oil Extract against Induced Topical Skin Infection by Candida albicans in Mice in vivo. Egyptian J. Hospital Med., 91(1): 3855-3861. https://dx.doi.org/10.21608/ejhm.2023.293468.

Al-Shammari S K, Al-Nouri D M, Arzoo S, Al-Harbi LN (2023). Effect of Different Nuts Oil Consumption on Morphological Features and Some Biomarkers of Inflammation in Adjuvant-Induced Arthritis (AIA) Rat Model. Appl. Sci., 13(5): 3318. https://doi.org/10.3390/app13053318.

Anvarinezhad M, Javadi A, Jafarizadeh-Malmiri H (2020). Green approach in fabrication of photocatalytic, antimicrobial, and antioxidant zinc oxide nanoparticles–hydrothermal synthesis using clove hydroalcoholic extract and optimization of the process. Green Process. Synth., 9(1): 375-385. https://doi.org/10.1515/gps-2020-0040.

Ayad Z M, Ibrahim OM S, Omar LW (2019). Biosynthesis and characterization of silver nanoparticles by Silybum marianum (silymarin) fruit extract. Adv. Anim. Vet. Sci., 7(2): 122-130. http://dx.doi.org/10.17582/journal.aavs/2019/7.2.122.130.

Bandyopadhyay S K (2018). Arthritis-a review on two types. J. Am. Ceram. Soc., 92: 1153-1177.

Cadau S, Gault M, Berthelemy N, Hsu C Y, Danoux, L, Pelletier N, ... & Pain S (2022). An Inflamed and Infected Reconstructed Human Epidermis to Study Atopic Dermatitis and Skin Care Ingredients. Int. J. Molecul. Sci., 23(21): 12880.

Choi S H, Won K J, Lee R, Cho H S, Hwang S H, Nah S Y (2021). Wound healing effect of gintonin involves Lysophosphatidic acid receptor/vascular endothelial growth factor signaling pathway in keratinocytes. Int. J. Molecul. Sci., 22(18): 10155.

Crowson C S, Matteson E L, Myasoedova E, Michet C J, Ernste F C, Warrington K J, ... & Gabriel S E (2011). The lifetime risk of adult-onset rheumatoid arthritis and other inflammatory autoimmune rheumatic diseases. Arthritis Rheumat., 63(3): 633-639. https://doi.org/10.1002/art.30155.

Elbestawy M K, El-Sherbiny G M, Moghannem S A (2023). Antibacterial, antibiofilm and anti-inflammatory activities of eugenol clove essential oil against resistant Helicobacter pylori. Molecules., 28(6): 2448.

El-Tanbouly G S, Abdelrahman RS (2022). Novel anti-arthritic mechanisms of trans cinnamaldehyde against complete Freund's adjuvant-induced arthritis in mice: involvement of NF-κB/TNF-α and IL-6/IL-23/IL-17 pathways in the immuno-inflammatory responses. Inflammopharmacology., 30(5), 1769-1780.

Faiq M K, Kadhim D J, Gorial F I (2019). The Belief about Medicines among a Sample of Iraqi Patients with Rheumatoid Arthritis. Iraqi J. Pharmaceut. Sci. (P-ISSN 1683-3597 E-ISSN 2521-3512), 28(2): 134-141.https://doi.org/10.31351/vol28iss2pp134-141.

Farhan H S, Al-Azizz S A, Al-Jadaan, S. A. (2023). Antibacterial and Antioxidant Activities of Syzygium Aromaticum Capparis Spinosa, and Some Novel Acylselenourea and Acylthourea Derivatives. Biomed. Chem. Sci., 2(2): 109-118.

Halasz H, Carpenter S (2022). Challenges and future directions for LncRNAs and inflammation. In Long Noncoding RNA: Mechanistic Insights and Roles in Inflammation (pp. 179-183). Cham: Springer International Publishing.

Hasan HF, Khazal KF, Luaibi OK (2014). The effect of crude alcoholic extract of Withania somnifera leaves in experimentally induced arthritis in mice. J.Thi-Qar Sci., 4 (2):45-51.

Hasan HF, Raheem SS (2021). Preparation of Poly (Lactic-co-glycolic Acid)-Loaded Pentoxyfilline by Nanoparticipation

Technique. Med. J. Babylon., 18(1): 12-17. https://doi.org/10.4103/MJBL.MJBL_59_20

Hosseini R, Ferns G A, Sahebkar A, Mirshekar M A, Jalali M (2021). Zinc supplementation is associated with a reduction in serum markers of inflammation and oxidative stress in adults: a systematic review and meta-analysis of randomized controlled trials. Cytokine., 138: 155396. https://doi.org/10.1016/j.cyto.2020.155396.

Husain W M, Araak J K, Ibrahim O M (2019). Green synthesis of zinc oxide nanoparticles from (Punica granatum L) pomegranate aqueous peel extract. Iraqi J. Vet. Med., 43(2): 6-14. https://doi.org/10.30539/iraqijvm.v43i2.524.

Husain W M, Kadhim J A, Ibrahim O M S (2019). Effect of different doses from znonps on the pituitary-testes axis function in adult male rats. Adv. Anim. Vet. Sci., 7(7): 550 556. https://doi.org/10.17582/journal.aavs/2019/7.7.550.556.

Ishaq A, Syed Q A, Khan M I, Zia M A (2019). Characterising and optimising antioxidant and antimicrobial properties of clove extracts against food-borne pathogenic bacteria. Int. Food Res. J., 26(4): 1165-1172.

Ismail C A N, Tan D C, Khir N A M, Shafin N (2022). A Review on Complete Freund's Adjuvant-Induced Arthritic Rat Model: Factors Leading to its Success. IIUM Medical J. Malaysia., 21(4).

Jayachandran A, Aswathy T R, Nair A S (2021). Green synthesis and characterization of zinc oxide nanoparticles using Cayratia pedata leaf extract. Biochem. Biophy. Rep., 26: 100995. https://doi.org/10.1016/j.bbrep.2021.100995.

Jiang J, Pi J, Cai J (2018). The advancing of zinc oxide nanoparticles for biomedical applications. Bioinorg. Chem. Applicat., 2018.

Katariya M, Singh J, Sirdesai A, Bandyopadhyay P (2023). In vitro assessment of babool extract, mint and clove oil containing toothpaste on gingivitis causing bacteria. J. Adv. Microbiol. Res., 4(1): 128-133. https://doi.org/10.22271/micro.2023.v4.i1b.86.

Khan M A, Anwar M F, Ahmad M (2023). Ocimum sanctum L water extract: In-situ green synthesis of zinc oxide nanoparticles for treatment of rheumatoid arthritis: Preclinical study. J. Trace Elements Med. Biol., 79: 127212. https://doi.org/10.1016/j.jtemb.2023.127212.

Li J, Wang P, Zhou T, Jiang W, Wu H, Zhang S, Wang H (2023). Neuroprotective effects of interleukin 10 in spinal cord injury. Front. Molecul. Neurosci., 16.

Liang Y, He J, Guo B (2021). Functional hydrogels as wound dressing to enhance wound healing. ACS nano., 15(8): 12687-12722. https://doi.org/10.1021/acsnano.1c04206.

Lopez-Miranda J L, Molina G A, González-Reyna M A, España-Sánchez B L, Esparza R, Silva R, Estévez M (2023). Antibacterial and Anti-Inflammatory Properties of ZnO Nanoparticles Synthesized by a Green Method Using Sargassum Extracts. Int. J. Molecul. Sci., 24(2): 1474 .https://doi.org/10.3390/ijms24021474.

Mahdi M F, Razzak N A A, Omer T N, Hadi M K (2012). Design and synthesis of possible mutual prodrugs by coupling of NSAIDs with sulfa drugs by using glycolic acid as spacer. Pharmacie Globale, 3(2): 1.

Mukherjee S (2023). A Study of Antibacterial and Anti Inflammatory activity Of Zinc Oxide Nanoparticles Znonps on multiple Antibiotic resistant Staphylococcus Aureus induced Inflammatory Rat model.

Naser N H, Mahdi M F, Omar T N, Fadhil A A (2011). Synthesis and preliminary pharmacological evaluation of new analogues of diclofenac as potential anti-inflammatory agents. Iraqi J. Pharm. Sci., 20(1): 25-32. https://doi.org/10.31351/vol20iss1pp25-32.

Omran R H, Zahra'a A A, Alrawi A A (2022). Evaluation of Some New Cytokines in Rheumatoid Arthritis. J. Facult. Med. Baghdad., 64(3): 159-162.

Parlindungan F, Hidayat R, Ariane A, Shatri H (2023). Association between Proinflammatory Cytokines and Anxiety and Depression Symptoms in Rheumatoid Arthritis Patients: A Cross-sectional Study. Clin. Pract. Epidemiol. Ment. Health. 19(1).

Qin H, Fu Y, Zhou K, Song H, Fang G, Chen Q, Pang Y (2023). Toddalia asiatica extract attenuates adjuvant-induced arthritis by modulating colon Th17/Treg balance and colony homeostasis. J. Ethnopharmacol., 313: 116542. https://doi.org/10.1016/j.jep.2023.116542.

Salih A N , Ibrahim O M , Eesa M J (2016). Biosynthesis of silver nanoparticles and evaluate its activity in promoting burns healing in rabbits. Al-Anbar J. Vet. Sci., 9(2): 47-58.

Salih A N, Ibrahim O M, Eesa M J (2017). Antibacterial activity of biosynthesized sliver nanoparticles against Pseudomonas aeruginosa in vitro. Iraqi J. Vet. Med., 41(1): 60-65.

Shaba E Y, Jacob J O, Tijani J O, Suleiman M A T (2021). A critical review of synthesis parameters affecting the properties of zinc oxide nanoparticle and its application in wastewater treatment. Appl. Water Sci., 11: 1-41. https://doi.org/10.1007/s13201-021-01370-z.

Singha L R, Das S, Das M K (2023). Plant-derived Anti-arthritic Nanomedicines for Effective Therapy in the Management of Inflammatory Diseases. Pharmacog. Rev., 17(33). https://doi.org/: 10.5530/097627870287.

Xu J, Huang Y, Zhu S, Abbes N, Jing X, Zhang L (2021). A review of the green synthesis of ZnO nanoparticles using plant extracts and their prospects for application in antibacterial textiles. J. Engineer. Fibers Fabrics., 16: 15589250211046242. https://doi.org/10.1177/15589250211046242