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Original Research Article

Pharmacological Effect of Eugenol on Freund's Adjuvants -Induced Arthritis in Experimental Rats

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Abstract: The objective of this experimental investigation was to examine the efficacy of eugenol. Against arthritis induced by complete Freund's Adjuvants (CFA) injected subcutaneously into the hind paw of the experimental rat model. Macroscopic clinical indication of Polyarthritis shows first erythema and edema in the hind paws of rat. Groups of this experiment were divided as follows, first groups was positive control while second and third groups received daily treatment with eugenol and dexamethasone orally via intragastric tube at doses (10 mg/ and 1mg/ Kg) respectively for 7 days. The initiation of treatment occurred on the day 7 from the day of induction of arthritis and continued up to day 14, at which point the rats were each sacrificed. The left and right paw volume variations were all measured on different days up to 14 days and after14 days, Tumor necrosis factor-α, Interleukin -10, and histopathological change were measured. Significant reduction ($P \leq 0.05$) in the development of edema in the hind paw was observed in the group treated with eugenol compared to dexamethasone and positive control groups. The histological examination revealed that the groups treated with eugenol had a normal articular cartilage and joint cavity, in contrast to the other group. Furthermore, eugenol maintained normal levels of the cytokine markers as inflammatory indicators, with significant difference from control groups. consequently, the results of the current investigation indicate that eugenol can be used as anti-inflammatory treatments and can be used as a beneficial adjuvant in the treatment of human arthritis.

Keywords: Rheumatoid arthritis (RA), eugenol, Freund's adjuvant.

INTRODUCTION

Rheumatoid arthritis, also referred to as (RA) is a persistent inflammatory condition that causes the deterioration of bone and cartilage caused by excessive growth of synovial tissue and the invasion of the inflammatory cells [1]. This inflammatory disease affects around 0.5-1% of the global population, with a reported frequency of 0.75% in India [2]. The exact cause of RA is still unclear, however it is thought that a mix of hereditary and environmental factors are significant contributors [3]. Pro-inflammatory cytokines are essential in the development of rheumatoid arthritis (RA) [4, 5]. Tumor necrosis factor-α (TNF- α), interleukin IL-6 and interleukin 1-β (IL-1β), are important pro-inflammatory cytokines that are known to contribute significantly to the inflammation seen in individuals with rheumatoid arthritis (RA). Prior studies have documented the presence of oxidative stress in individuals with rheumatoid arthritis (RA), which can result from either excessive generation of free radicals or a compromised anti-oxidant defense mechanism [3]. The treatment approach for RA entails the administration of non-steroidal anti-inflammatory medicines (NSAIDs) and the disease modifying antirheumatic drugs (DMARDs). These medications offer symptomatic relief and also affect the progression of the illness. Additionally, the use of immunosuppressants can lead to cardiovascular problems, gastrointestinal ulcers, and the development of opportunistic infections [6]. Several natural substances, have gained significant interest in recent decades due to their proven safety and effectiveness. A novel therapy approach utilizing natural compounds has been developed due to the adverse effects and limited effectiveness of existing the anti-inflammatory and immunosuppressive medications for rheumatoid arthritis [3].

Eugenol, specifically 4-allyl-2-methoxyphenol, is the primary constituent found in the essential oil of several aromatic plants, such as clove (Zyzygium aromaticum). It is recognized for its analgesic, antioxidant and neuroprotective effects, among other beneficial qualities [7, 8]. Furthermore, eugenol and similar substances demonstrate anti-inflammatory properties, such as the prevention of lipopolysaccharide (LPS)-induced activation of nuclear factor-kappa B (NF-κB), release of cytokines, and production of cyclooxygenase-2 (COX-2) by macrophages in the vitro environment [9] and suppression of the 5-lipoxygenase expression in the polymorphonuclear cells [10]. Although eugenol has been shown to have anti-inflammatory properties, there is just few documented instance of it demonstrating antirheumatic effects in the model of inflammatory arthritis [11]. The present investigation assessed the impact of eugenol in Freund's complete adjuvant induced arthritis (CIA) in rat model compared with dexamethasone as alternative antiinflamatory medicine.

MATERIAL AND METHODS

Freund's complete adjuvant was procured from (Santa cruz biotechnology/USA). Eugenol (99%) was purchased from (Sigma, USA). All other substances utilized in this investigation were of analytical quality.

Animals: This study was conducted on Eighteen male rats with 200–250 g BW, were gotten from the animal house of veterinary Medicine/ Department of Pharmacology, University of Baghdad /Iraq. Every rat was provided with unrestricted access to water and a regular rodent feed.

Experimental Design

Animals were randomly divided into three groups of six animals each. Arthritis was generated by injecting 0.1ml of the complete Freund's adjuvant(CFA) intradermally into the planter area of the right hind-paw on day 0 (12,13). Rats were given a booster injection on day 4 and then were observed on each day to detect any indications of arthritis. Group A, which serve as normal controls rats were received distill water only (2ml/ Kg B.W./ day). Group B, rats receive eugenol (orally via intragastric tube)(10 mg/ Kg B.W./ day) for 14 days. Group C, rats receive Dexamethasone orally (1mg/Kg. B.W./ day) for 14 days. The treatment started on day 7 and persisted until day 14, at which point the rats were euthanized. The dosage of eugenol was determined based on our early research and in vivo experiments that have shown its anti-inflammatory properties [14-18].

Parameters

1. The changes in paw volume will be observed on many days, up to 14 days after the injection of Freund's adjuvant.
2. Measuring Tumor necrosis factor (TNF-α) and Interleukin (IL)-10 following Freund's adjuvant injection before treatment and at 14 days after treatment.
3. Histopathological Examination of joint after 7 days following Freund's adjuvant injection before treatment and at day 14 after treatment.

Measurement of Hind Paw Volumes

The volumes of the right hind-paw of rats were put according to the volume displacement by use of the vernier caliper. Once before the injection of the complete Freund's adjuvant (CFA). The average volume (Vo) of the right hind-paw of each rat was computed from 5 readings and it was obtained after injection of complete Freund's adjuvant readings (Vt) for each rat. Edema was incrementally expressed in the volume of paw (19). Measuring the changes of paw volume had done on different days (7 days after induction and at 14 days after treatment). On the day 14, rats will be anesthetized with diethyl ether and to assure the induction of arthritis, it is necessary to remove edematous tissue from the injected hind-paw.

Histopathological Studies

Rats have been euthanized and joint tissue biopsies have been taken. The tissues were washed with PBS and fixed in 10% neutral buffered formalin and covered with gauze. After fixation, samples will be dehydrated using ethyl alcohol at various concentrations (70, 80, 90, and 100% twice each) for two hours before being cleaned with xylene for half an hour. Blocks of paraffin are produced by infiltrating samples with paraffin wax at 58–60 C0 and then embedding them in fresh paraffin wax. Using a rotary microtome, 5–6 um thick sections were cut, stained with hematoxylin and eosin, and examined under a light microscope [20].

Blood Collection

Blood samples (2 ml) were collected from eye by optic vein puncture technique without anticoagulant test tube which was centrifuged at 3000 rpm (10 minutes), then the serum were gathered in 2 ml eppendrof tubes which was labeled for date and time of blood drawing and freezing at -8 C0 till use.

Measurement of IL10 and TNF-α Concentration in rat serum: ELISA assay was achieved according to the method described by the manufacturing company (Cloudy-Clone /USA).

Statistical-Analysis

The SAS (2018) software was utilized to identify the impact of various factors on research parameters using statistical analysis. The study utilized the least significant difference (LSD) test, which is a method of analysis of variation (ANOVA-1), to compare means and determine significant differences.

RESULTS

This study demonstrates the effectiveness of the treatment with eugenol in reducing arthritis symptoms in experimental rats models. After injection of Complete Freund's adjuvant entire hind paw, extending to the ankle, causing swells progressively and erythema, which peaked on the day 7. It was evident that paw edema in control (not treated) inflamed paw was increased while eugenol and dexamethasone treated groups were significantly suppressed the development of the joint-swelling caused by Freund's adjuvant injection after 7 days of starting treatment in the paw edema induced by complete freund's adjuvant (FCA) (Figure 1).

Freund’s complete adjuvant-induced swelling was entirely inhibited with no further development seen in treated groups. The medication therapy administered for a duration of 7 days, starting from day 7 after the adjuvant injection, effectively inhibited the subsequent rise in edema of the injected ankle that associated with the onset of arthritis. The results in the Table (1) shown the inhibition of swelling formation with eugenol and dexamethasone treated groups during 14 days. In addition, the inhibition of swelling were significantly different (P≤0.05) during the 14 days.

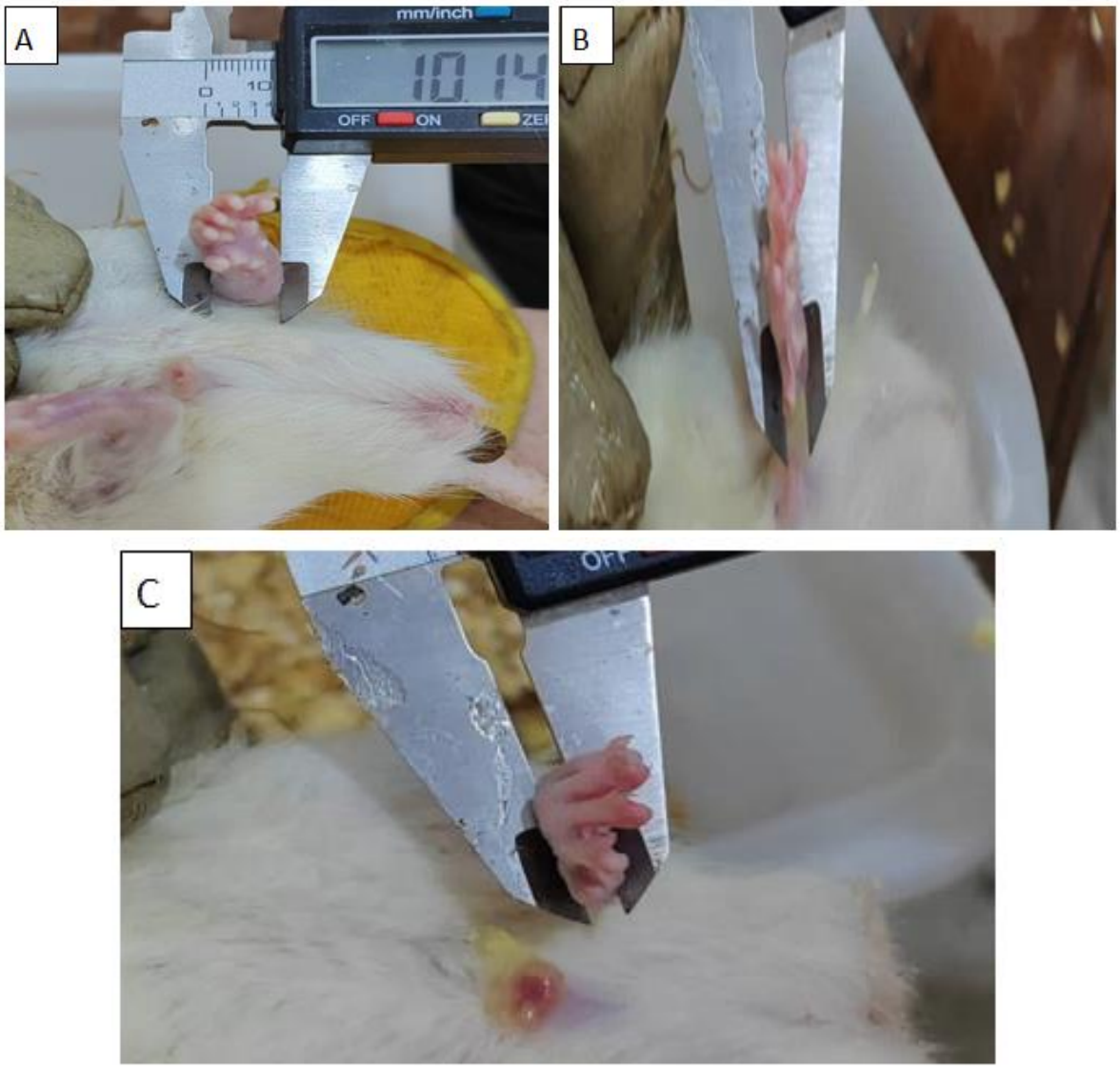

Figure 1: Hind paw oedema induced by Freund's Adjuvants in rats: Measurement paw oedema in A: Positive control-after 7 days, B: Treat with Eugenol after 14 days, C: Treat with Dexamethasone after 14 days

Table 1: The thickness of inflammation in the injected foot corresponds to the rise in paw edema observed in arthritic rats 14 days after arthritis induction treated with Eugenol, Dexamethasone compared to the positive control group

Groups	Zero	Day 7	Day 14
Positive Control	3.54±0.12 A c	5.64±0.19 A b	8.40±0.08 A a
Treat with eugenol	3.50±0.11 A a	3.11±0.16 B b	2.93±0.08 C c
Treat with dexamethason	3.63±0.07 A a	3.08±0.05 B b	2.78 ±0.07 B c
LSD	**0.02**		

Different capital letters in same column and small letters in same row denote to the significant difference at P<0.05

Complete Freud's adjuvant (CFA) induced arthritis in male Wistar albino rats as evidenced by the presence of increased levels of the inflammatory cytokines in the serum of the injected rats. The serum levels of interleukin-10 were quantified in picograms per milliliter for each group and are presented in a Table (2). Prior to induction, there were no significant distinctions (P > 0.05) among the various groups. However, on day 14 following treatment with eugenol, IL-10 concentrations in the serum experienced a significant increase (P≤0.05) compared to the other groups. Furthermore, the groups that were received dexamethasone exhibited an normal level of IL-10 after 14 days, although it remained significantly higher than the positive control group, which showed no improvement in serum levels of the anti-inflammatory cytokine IL-10.

Table 2: The effectiveness of eugenol, dexamethasone on the serum level of IL-10 after day 14 of injecting rats with Freund's adjuvant compared with positive control group

Groups	IL- 10 (Pg/ml)	
	Before Induction on day 0	**After day 14**
Positive Control group	110.54 ±2.15 A a	50.82±5.37 C b
Treat with eugenol group	109.92±2.18 A b	130.98 ±6.21 A a
Treat with dexametha one group	101.42±3.12 A a	107.29±6.24 B a
LSD value	**3.13**	

Different capital letters in same column and small letters in same row denote to the significant difference at P<0.05

The levels of serum TNF-α in each group at day fourteen after injecting rats with Freund's adjuvant are shown in Table (3), According to the study's findings, TNF-α in the treated group was not statistically significant (P>0.05) when compared to the level before induction, while TNF-α concentrations in the positive control groups were considerably higher (P≤0.05) when compared to its level before induction.

Table 3: The effectiveness of eugenol, dexamethasone on the serum level of TNF-α after day 14 of injecting rats with Freund's adjuvant compared with positive control group

Groups	TNF-α (Pg/ml)	
	Before induction on day 0	**After day 14**
Positive Control group	318.54±2.89 A a	509.70±6.38 A b
Treat with eugenol group	320.87±4.88 A a	350.54±2.98 B a
Treat with dexamethasone group	321.0 ±3.33 A a	377.26 ±3.90 C a
LSD value	**1.84**	

Different capital letters in same column and small letters in same row denote to the significant difference at P<0.05

Microscopically Changes

The histopathological section of knee joint in control group after14 days of rheumatoid arthritis induction showed intense inflammation of the joints which characterized by thickening of joint capsule associated with congestion and infiltration of specific type of white blood cells (MNCs) and necrosis of collagen fibers (Figure 2).

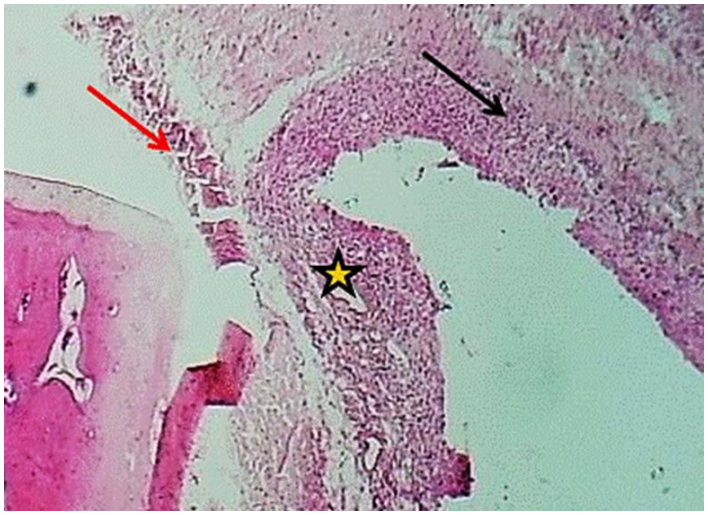

Figure 2: Joint section (after induction of arthritis on day 7) shows: of thickening of joint capsule (Asterisk) also showed congestion accompanying with infiltration of MNCs (Black arrow) & necrosis of collagen fibers (Red arrows). H&E stain. 100x

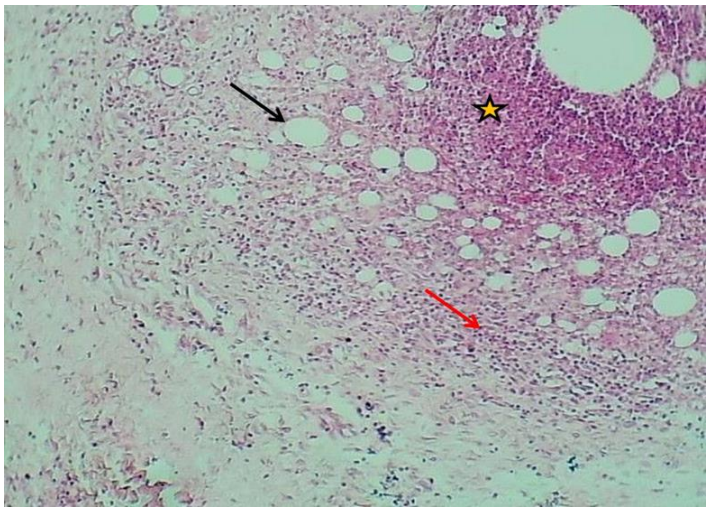

Figure 3: Joint section (control positive) after induction without treatment on day 14 shows: focal granulomatous lesion within joint capsule (Asterisk), edema (Black arrow) & infiltration of MNCs (Black arrow). H&E stain. 100x.

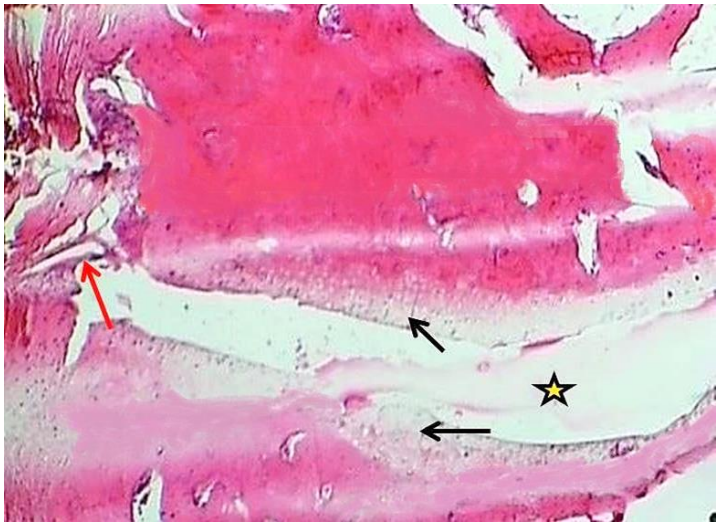

Figure 4: Joint section (treated with eugenol on day14 after induction of arthritis) shows: normal joint cavity (asterisk), normal articular cartilage (Black arrows), and normal joint capsule (Red arrow). H&E stain. 40x

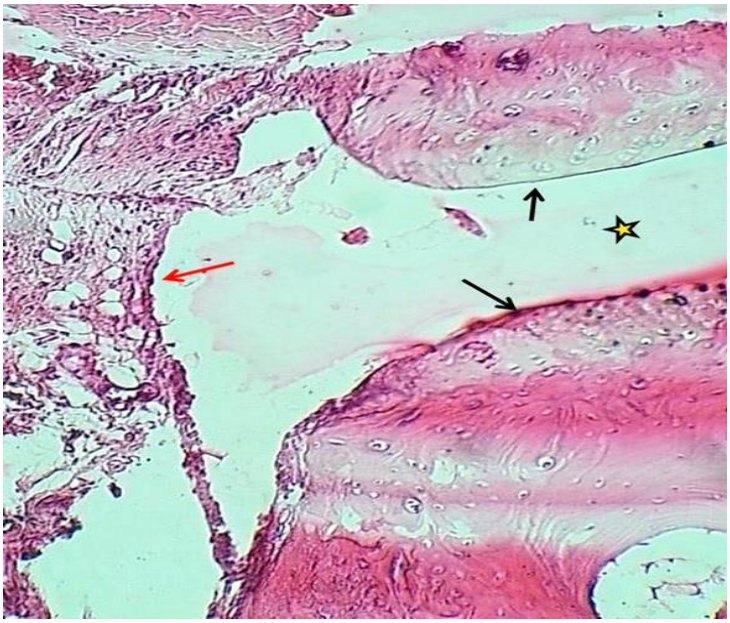

Figure 5: Joint section (treated with dexamethasone on day 14 after induction of arthritis) shows: normal joint cavity (Asterisk), normal articular cartilage (Black arrows) & inner synovial membrane (Red arrow). H&E stain. 100x

The histological examination of the knee joint in the groups treated with eugenol and dexamethasone after 14 days of arthritis induction revealed that joint cavity seems normal, with intact articular cartilage and a healthy synovial membrane. However, the outer fibrous capsule shows slight thickening due to infiltration of mononuclear leukocytes (MNCs), angiogenesis and fibroplasia (Figure 4 & 5) compared to the control group of the trial that did not receive any treatment for 14 days, which showed severed arthritis that characterized by focal granulomatous lesion within joint capsule, edema infiltration of MNCs (Figure 2 & 3).

DISCUSSION

Many studies have demonstrated that eugenol, a component found in plant essential oils, possesses anti-inflammatory, antioxidant, DNA-protective, antibacterial and analgesic activities [7, 8]. Previous investigation suggests that Syzygium aromaticum, which contains eugenol as its primary component, exhibits an immunomodulatory impact [21]. The objective of this study was to investigate the potential impact of eugenol on an existing arthritic condition in male rats. Experimental models have provided limited progress in understanding the causes of human autoimmune disorders. However, they do present a chance to explore novel treatment approaches for enhancing joint inflammation. The use of adjuvant-induced arthritis in rats has been authorized as an experimental model for investigating the development of rheumatoid arthritis (RA) [22, 23].

This study examined the impact of eugenol on the levels ofIL-10 and TNF-α in the affected joints, considering that cytokines have a role in both cell-to-cell communication and the tissue destruction that seen in rheumatoid arthritis [24]. The prevalence that pro-inflammatory cytokines over the anti-inflammatory cytokines has also been documented in humans with rheumatoid arthritis (RA) and animals with arthritis, as a result of an imbalance between the cytokines released by Th1 and Th2 cells [25-27]. These pro-inflammatory cytokines are also produced by macrophages and the synovial lining cells. TNF- α is regarded as the primary cytokine that enhances the production of reactive oxygen species and induces cartilage degradation in animal models with arthritis and in individuals with rheumatoid arthritis [28-30], since this cytokine is involved in bone resorption and cartilage remodeling by promoting the development of osteoclast cells and inhibiting collagen production. On the other hand IL-10 is the primary immunoregulatory cytokine which is released by T cells. It suppresses the synthesis of chemokines, pro-inflammatory cytokines, and matrix proteases in the macrophages.

In the present study, eugenol reduced the increasing productions of TNF-α levels in the adjuvant-induced arthritis in male rats. Thus, we propose that eugenol may have potential in the therapeutic management of autoimmune rheumatoid arthritis and this agree with the study of [11, 2]. The anti-inflammatory properties of eugenol were observed as a result of its capacity to prevent the release of pro-inflammatory cytokines and promote the release of anti-inflammatory IL-10, consequently managing the balance between Th1 and Th2 responses. The histopathological study also verified that eugenol effectively improved inflammation and reduced bone degradation. Therefore, eugenol may preserve bone and cartilage by suppressing the synthesis of pro-inflammatory cytokines.

Prior research has further demonstrated the immunomodulatory, anti-inflammatory and analgesic properties of eugenol in treated inflammatory conditions in animal models [31, 16, 32, 33]. Eugenol acts as an anti-inflammatory agent by suppressing the formation of prostaglandins and the neutrophil chemotaxis. Moreover, scientific evidence has demonstrated that phenolic antioxidants have the ability to impede the activation of cyclooxygenase (COX)-2 in lipopolysaccharide (LPS) activated macrophages. This, in turn, can diminish the abundance of inflammatory cells and therefore decrease the thickness of skin folds [34].

Many studies have shown that eugenol can control the functions of macrophages that negatively regulate inflammation since they are immune cells that involved in the secretion of mediators (like nitric oxide and pro-inflammatory cytokines), which considered crucial to vascular and cellular events throughout the progression of the inflammatory process [35]. On the other hand, eugenol functions as a natural medication because it contains a number of essential vitamins and minerals that enabling it enhances blood circulation, so assuring a greater supply of nutrients and oxygen to the affected region [36].

Overall, the findings of this study provide further insight into the previously reported advantageous anti-inflammatory properties of eugenol. These results indicate that eugenol might potentially serve as an alternate or supplementary therapy for chronic inflammatory conditions like rheumatoid arthritis.

CONCLUSION

The present study observed that eugenol effectively reduced the level of severity of arthritis in rats, as evidenced by the reduction in hind paw swelling and a decrease in inflammatory markers such as IL10 and TNF-α. Subsequent research might investigate the preventative and properties of eugenol in relation to rheumatoid arthritis. We believe that our findings will make a valuable contribution to the therapeutic applications in the management and treatment of rheumatoid arthritis. However, further research is necessary to have a comprehensive understanding of the mechanism by which eugenol operates.

REFERENCES

1. Smolen, J. S., & Aletaha, D. (2009). Developments in the clinical understanding of rheumatoid arthritis. *Arthritis Research & Therapy*, *11*, 1-9.
2. Mateen, S., Shahzad, S., Ahmad, S., Naeem, S. S., Khalid, S., Akhtar, K., ... & Moin, S. (2019). Cinnamaldehyde and eugenol attenuates collagen induced arthritis via reduction of free radicals and pro-inflammatory cytokines. *Phytomedicine*, *53*, 70-78.
3. Mateen, S., Zafar, A., Moin, S., Khan, A. Q., & Zubair, S. (2016). Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. *Clinica chimica acta*, *455*, 161-171.
4. Kroemer, G., & Martínez-A, C. (1991). Cytokines and autoimmune disease. *Clinical immunology and immunopathology*, *61*(3), 275-295.
5. Veselinovic, M., Barudzic, N., Vuletic, M., Zivkovic, V., Tomic-Lucic, A., Djuric, D., & Jakovljevic, V. (2014). Oxidative stress in rheumatoid arthritis patients: relationship to diseases activity. *Molecular and cellular biochemistry*, *391*, 225-232.
6. Yeom, M. J., Lee, H. C., Kim, G. H., Lee, H. J., Shim, I., Oh, S. K., ... & Hahm, D. H. (2006). Anti-arthritic effects of Ephedra sinica STAPF herb-acupuncture: inhibition of lipopolysaccharide-induced inflammation and adjuvant-induced polyarthritis. *Journal of Pharmacological Sciences*, *100*(1), 41-50.
7. Park, S. H., Sim, Y. B., Lee, J. K., Kim, S. M., Kang, Y. J., Jung, J. S., & Suh, H. W. (2011). The analgesic effects and mechanisms of orally administered eugenol. *Archives of pharmacal research*, *34*, 501-507.
8. Yogalakshmi, B., Viswanathan, P., & Anuradha, C. V. (2010). Investigation of antioxidant, anti-inflammatory and DNA-protective properties of eugenol in thioacetamide-induced liver injury in rats. *Toxicology*, *268*(3), 204-212.
9. Murakami, Y., Shoji, M., Hirata, A., Tanaka, S., Yokoe, I., & Fujisawa, S. (2005). Dehydrodiisoeugenol, an isoeugenol dimer, inhibits lipopolysaccharide-stimulated nuclear factor kappa B activation and cyclooxygenase-2 expression in macrophages. *Archives of biochemistry and biophysics*, *434*(2), 326-332.
10. Raghavenra, H., Diwakr, B. T., Lokesh, B. R., & Naidu, K. A. (2006). Eugenol—the active principle from cloves inhibits 5-lipoxygenase activity and leukotriene-C4 in human PMNL cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *74*(1), 23-27.
11. Grespan, R., Paludo, M., de Paula Lemos, H., Barbosa, C. P., Bersani-Amado, C. A., de Oliveira Dalalio, M. M., & Cuman, R. K. N. (2012). Anti-arthritic effect of eugenol on collagen-induced arthritis experimental model. *Biological and Pharmaceutical Bulletin*, *35*(10), 1818-1820.
12. 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 Journal Malaysia*, *21*(4).

13. El-Tanbouly, G. S., & Abdelrahman, R. S. (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.
14. Abd El Motteleb, D. M., Selim, S. A., & Mohamed, A. M. (2014). Differential effects of eugenol against hepatic inflammation and overall damage induced by ischemia/re-perfusion injury. *Journal of immunotoxicology*, *11*(3), 238-245.
15. 15-Babu, P. S., Prabuseenivasan, S., & Ignacimuthu, S. (2007). Cinnamaldehyde—a potential antidiabetic agent. *Phytomedicine*, *14*(1), 15-22.
16. Ferland, C. E., Beaudry, F., & Vachon, P. (2012). Antinociceptive effects of eugenol evaluated in a monoiodoacetate-induced osteoarthritis rat model. *Phytotherapy research*, *26*(9), 1278-1285.
17. Srinivasan, S., Sathish, G., Jayanthi, M., Muthukumaran, J., Muruganathan, U., & Ramachandran, V. (2014). Ameliorating effect of eugenol on hyperglycemia by attenuating the key enzymes of glucose metabolism in streptozotocin-induced diabetic rats. *Molecular and cellular biochemistry*, *385*, 159-168.
18. Zakaria, S., Mahmoud, A. A., Hasan, R. A., Mahmoud, M. F., & El-Fayoumi, H. M. (2016). Cinnamaldehyde mitigates carbon tetrachloride-induced acute liver injury in rats through inhibition of toll-like receptor 4 signaling pathway.
19. Najim, S. M., Abd, M. R., Fadhil, A. A., & Hassan, A. F. (2023). Fimasartan attenuates edema and systemic changes in egg albumin-induced paw inflammation in rats. *Journal of Advanced Pharmacy Education & Research*, *13*(1).
20. Suvarna, S. K. (Ed.). (2016). *Atlas of adult autopsy: A guide to modern practice*. Springer.
21. Carrasco, F. R., Schmidt, G., Romero, A. L., Sartoretto, J. L., Caparroz-Assef, S. M., Bersani-Amado, C. A., & Cuman, R. K. N. (2009). Immunomodulatory activity of Zingiber officinale Roscoe, Salvia officinalis L. and Syzygium aromaticum L. essential oils: evidence for humor-and cell-mediated responses. *Journal of Pharmacy and Pharmacology*, *61*(7), 961-967.
22. 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. *Journal of Ethnopharmacology*, *313*, 116542.
23. Al-Shammari, S. K., Al-Nouri, D. M., Arzoo S., & Al-Harbi, L. N. (2023). Effect of Different Nuts Oil Consumption on Morphological Features and Some Biomarkers of Inflammation in Adjuvant-Induced Arthritis (AIA) Rat Model. *Applied Sciences*, *13*(5), 3318.
24. Dong, J., Gao, Y., Liu, Y., Shi, J., Feng, J., Li, Z., ... & Yang, G. (2010). The Protective Antibodies Induced by a Novel Epitope of Human TNF-α Could Suppress the Development of Collagen-Induced Arthritis. *PloS one*, *5*(1), e8920.
25. Brennan, F. M., & McInnes, I. B. (2008). Evidence that cytokines play a role in rheumatoid arthritis. *The Journal of clinical investigation*, *118*(11), 3537-3545.
26. Sharma, S., Sahu, D., Das, H. R., & Sharma, D. (2011). Amelioration of collagen-induced arthritis by Salix nigra bark extract via suppression of pro-inflammatory cytokines and oxidative stress. *Food and chemical toxicology*, *49*(12), 3395-3406.
27. Umar, S., Zargan, J., Umar, K., Ahmad, S., Katiyar, C. K., & Khan, H. A. (2012). Modulation of the oxidative stress and inflammatory cytokine response by thymoquinone in the collagen induced arthritis in Wistar rats. *Chemico-biological interactions*, *197*(1), 40-46.
28. Ahmad, S., Alam, K., Hossain, M. M., Fatima, M., Firdaus, F., Zafeer, M. F., ... & Nafees, K. A. (2016). Anti-arthritogenic and cardioprotective action of hesperidin and daidzein in collagen-induced rheumatoid arthritis. *Molecular and cellular biochemistry*, *423*, 115-127.
29. Choy, E. H., & Panayi, G. S. (2001). Cytokine pathways and joint inflammation in rheumatoid arthritis. *New England Journal of Medicine*, *344*(12), 907-916.
30. 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. *Clinical Practice and Epidemiology in Mental Health*, *19*(1).
31. Bezerra, D. P., Militão, G. C. G., de Morais, M. C., & de Sousa, D. P. (2017). The Dual Antioxidant/Prooxidant Effect of Eugenol and Its Action in Cancer Development and Treatment. *Nutrients*, 9, 1367.
32. Kang, L. L., Zhang, D. M., Ma, C. H., Zhang, J. H., Jia, K. K., Liu, J. H., ... & Kong, L. D. (2016). Cinnamaldehyde and allopurinol reduce fructose-induced cardiac inflammation and fibrosis by attenuating CD36-mediated TLR4/6-IRAK4/1 signaling to suppress NLRP3 inflammasome activation. *Scientific Reports*, *6*(1), 27460.
33. Lee, S. C., Wang, S. Y., Li, C. C., & Liu, C. T. (2018). Anti-inflammatory effect of cinnamaldehyde and linalool from the leaf essential oil of Cinnamomum osmophloeum Kanehira in endotoxin-induced mice. *Journal of food and drug analysis*, *26*(1), 211-220.
34. Das Chagas Pereira de Andrade, F., & Mendes, A. N. (2020). Computational analysis of eugenol inhibitory activity in lipoxygenase and cyclooxygenase pathways. *Scientific reports*, *10*(1), 16204.
35. Arango Duque, G., & Descoteaux, A. (2014). Macrophage cytokines: involvement in immunity and infectious diseases. *Frontiers in immunology*, *5*, 117833.
36. Batiha, G. E. S., Alkazmi, L. M., Wasef, L. G., Beshbishy, A. M., Nadwa, E. H., & Rashwan, E. K. (2020). Syzygium aromaticum L.(Myrtaceae): traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. *Biomolecules*, *10*(2).