Biomedical & Pharmacology Journal, September 2024. Vol. **17**(3), p. 1837-1845

The Effect of An Aquatic Extract of *Eucalyptus globulus* Leaves on Reducing the Inflammation Parameters Caused by Carrageenan in Male Wistar Rats

Mustafa K. Mushatet[1]*, Asaad Abbas Khalaf[1], Doaa A. Hamad[1] and Thikra Abd Jary[2]

[1]University of Kerbala, College of Nursing, Branch of basic sciences, Kerbala, Iraq.
[2]University of Al-Ameed, College of Medicine, Branch of Biochemistry, Kerbala, Iraq.
*Corresponding Author E-mail: mustafa.k@uokerbala.edu.iq

https://dx.doi.org/10.13005/bpj/2989

(Received: 17 August 2024; accepted: 16 September 2024)

This research looked at how an aquatic *Eucalyptus globulus* extract (ECP) affected inflammation and oxidative stress in male rats that were given an experimental form of carrageenan (CRG). The study randomly assigned twenty-four male Wistar rats. In the study, 2 ml of physiological solution was administered to the control group, 0.6 g/kg of ECP BW was given to the ECP group orally, 100 ìl of the CRG solution dissolved in 1 milliliter of distilled water was injected intraperitoneally to the CRG group for 30 days, and 0.6 g/kg of BW of ECP was given orally to the C&E group for 15 days after 15 days of 100 ìl of the carrageenan solution dissolved in 1 milliliter of distilled water intraperitoneal injection. We sacrificed the rats after 30 days and analyzed the serum samples to measure white blood cell count (WBC), lymphocytes, monocytes, neutrophils, C-reactive protein (CRP), and tumor necrosis factor (TNF). The study found that the CRG and C&E groups had higher levels of WBC, lymphocytes, monocytes, neutrophils, CRP, and TNF than the ECP and control groups. The decrease in inflammation markers CRP and TNF in the serum of E&C group rats demonstrates that ECP's aqueous extract has anti-inflammatory properties. However, further clinical and histological research is necessary to validate its potential as a therapeutic agent.

Keywords: Carrageenan, C- reactive protein, *Eucalyptus globulus*, inflammatory, lymphocytes, monocytes, necrosis factor, neutrophils, and tumor.

In this study, we investigated the anti-inflammatory properties of an aquatic extract of *Eucalyptus globulus* (ECP) by inducing inflammatory indices using carrageenan._Herbal products are essential sources of herbal extracts and other pharmacological agents. Since ancient times, people have used the leaves of various *Eucalyptus* species as traditional medicines to treat fever, colds, and other ailments[1].

ECP leaves are utilized locally to treat burns, dermatitis, scabies, tonsillitis, and dysentery; they are also taken orally to treat articular pain, tonsillitis, dysentery, influenza, and cystitis. Research has shown that using ECP leaves orally can reduce cold symptoms during flu-episodes[2,3]. *Eucalyptus globulus* possesses remarkable pharmacological activities, including antioxidant and anti-inflammatory effects[4]. The naturally oils

in *Eucalyptus globulus* include 1-eucalyptol, which has a variety of pharmacological actions, including insecticidal, gastrointestinal, dermatological, anti-inflammatory, analgesic, antidiabetic, antioxidant, anticancer, and antibacterial properties[5–7].

Concisely, *Eucalyptus* leaves have the ability to protect the liver from damage and reduce oxidative stress due to reactive oxygen species (ROS) produced during metabolism[8], It has previously been shown to be a source of bioactive chemicals, namely phenolic compounds like phenolic acids, flavonoids, or hydrolyzable tannins. *E. globulus* leaves have long been used to treat respiratory issues[9,10].

Eucalyptus leaves have antioxidant properties, as evidenced by their ability to scavenge free radicals, activate antioxidant enzymes, and suppress inflammation by inhibiting lipoxygenase and lowering nitric oxide (NO) levels[10]. It was demonstrated that their ethanolic extracts reduced the levels of pro-inflammatory mediators and nitric oxide (NO)[11], and that they had the ability to scavenge free radicals[12].

A basic defense mechanism of the host in reaction to damaging stimuli such as, illness, and/or irritation is inflammation[13]. The symptoms of acute inflammation manifest suddenly and include edema, leukocyte emigration, enhanced vascular permeability, and vasodilation[14]. A series of cytokines governs the inflammatory process to a significant extent and is essential for cell-to-cell contacts and communications[15].

Carrageenan (E407) is a polysaccharide extracted from red algae (Chondrus crispus) that induces inflammation induced inflammatory in the rat16,17, Acute inflammation leads to the development of biphasic inflammatory responses in the tissue of the paw18. The main objective of the current research is to ascertain the therapeutic properties of *Eucalyptus* leaves and their role in mitigating the inflammatory activity of carrageenan.

Research design and methods

The current study aims to identify the therapeutic qualities of eucalyptus leaves and how they contribute to reducing carrageenan's inflammatory activity. We obtained 24 male Albino rats, aged 12 to 14 weeks, from the Animal House, College of Pharmacy / University of Karbala. The Institutional Animal Ethics Committee, University of Kerbala, Iraq, assisted in reviewing and approving all animal experimentation protocols, with the approval of experimental research. Animal maintenance therapies, as instructed by animal care experts, adhere to the International Animal Care and Use Guidelines[19]. We randomly divided these rats into four groups, each containing six rats, kept them in a separate cage, and gave them two weeks to acclimate to the water and food supplies, following the American Institute of Nutrition's semi-purified diet (AIN).

1. Group 1: control, which receives an oral dose of 2 ml of a normal saline physiological solution for 30 days.
2. Group 2: is the positive control¡ receiving 0.6 g/kg of *Eucalyptus globulus* extract (ECP) orally at a dose of 2 ml for 30 days. The (LD… €) of *eucalyptus* oil is 2770-4500 mg/kg (rat)[20].
3. Group 3: For 30 days, the Carrageenan group will receive an intraperitoneal injection of 100 ìl of the carrageenan solution (CRG) dissolved in 1 milliliter of distilled water.
4. Group 4: CRG and ECP Group (C&E): For fifteen days, this group receives an intraperitoneal injection of 100 ìl of the carrageenan solution dissolved in 1 milliliter, followed by an oral dose of ECP after two hours, to follow up on the inflammation status in the C&E group, in which inflammation was decreasing compared to the CRG group, in which inflammation was ascending (inflammation is directly proportional to the time of carrageenan injection) in the same period, which 30 days. We assumed that a period of 15 days is sufficient to stimulate an inflammatory state using intraperitoneal carrageenan[21], followed by an equal period of treatment using ECP to try to restore inflammatory factors to their normal levels as in the control group.

Following a 12-hour fast, we sacrificed the rats after 30 days and extracted seven ml of blood directly from the heart for a complete blood count (CBC). We recorded the results and separated the blood serum using an Inflammation Panel 1 Rat Kit to measure the percentage of oxidants, antioxidants, and inflammation. We assessed and contrasted the proportion of inflammation in the treated group with that of the control group.

Plant Materials Collection and Extraction Procedure

We collected the *Eucalyptus globulus*

leaves from our garden in Karbala, Iraq, in February 2024. The National Herbarium. We cut and divided the plant leaves into small pieces, dried them in the air for a few days, and ground them to powder using a motorized grinder (beerfingo, SR01, Algeria). We used a weighing machine (Suyue, China) to powder the plant materials into 250 g, which we then submerged in 750 ml of distillation water in airtight containers for three days. We then filtered the powder through a fresh cotton bed and No. 2 filter paper, respectively. We stored the crude extracts at 25 °C in well-closed containers for future use.

Devices and substances used in the study

To prepare Carrageenan, type IV (Sigma, USA), dissolve 0.01 g in 1 ml of distilled water. We measured the white blood cell (WBC) leucocyte, lymphocyte, monocyte, and neutrophil count by used BC-3000 hematology machines from Mindray (India). We used Rat CRP detection kits from Chondrex (USA) to measure the C-reactive protein (CRP) and a Rat TNF enzyme-linked immunosorbent assay kit (ELISA) for the TNF assay.

Data analysis

We display each result as the mean and standard deviation. ANOVA analysis was utilized to determine the experimental data's statistical significance, with a significance threshold of P <.05We used the Excel 2013 program as a numerical instrument to carry out this statistical study.

RESULTS

Tables 1 and 2 displays the noteworthy variations in blood parameter values between groups. Carrageenan has been altering the levels of WBC, lymphocytes, monocytes, neutrophils, CRP, and TNF. The levels of monocytes, neutrophils, CRP, and TNF were all the same between the ECP and the control groups (P = 0.05), but there was a remarkable rise (P< 0.05) between the CRG and the C&E group in contrast to the group of control. Carrageenan has caused significant Inflammation in the CRG group. The table also clearly showed that Carrageenan impacted WBC, lymphocytes, monocytes, neutrophils, CRP, and TNF. The CRG group showed a remarkable rise in contrast to the control and ECP groups. This result aligns with numerous previous studies[22,23]. This is due to the inflammation induced by carrageenan in the experiment animals[24,25], based on the findings of previous studies[22,23,25]. Additionally, Table 1 demonstrated that there was a considerable rise in the ECP group's WBC count as a result of ECP

Table 1. Evaluation of Blood parameters in different experimental configurations

Groups	WBC ($x10^3/\mu L$)	Lymphocytes ($x10^3/\mu L$)	Monocytes ($x10^3/\mu L$)	Neutrophils ($x10^3/\mu L$)
Control	04.00 ± 0.00	07.08 ± 0.15	00.25 ± 0.03	01.55 ± 0.19
ECP	04.85 ± 0.31 [a]	07.36 ± 0.20 [a]	00.20 ± 0.02	01.63 ± 0.09
CRG	13.50 ± 0.98 [ab]	11.10 ± 0.13 [ab]	01.97 ± 0.08 [ab]	04.77 ± 0.12 [ab]
C&E	08.83 ± 0.75 [ab]	09.42 ± 0.09 [ab]	01.16 ± 0.08 [ab]	03.79 ± 0.09 [ab]

The values are mean ± SD value, n = six in each group, [a] show the difference in statistics. With a control group, [b] statistical disparity according to illness group, (P < 0.05). ECP: Group of aqueous extract of *Eucalyptus globulus* leaves, CRG: group of Carrageenan, C&E: group of carrageenan then aqueous extract of *Eucalyptus globulus* leaves.

Table 2. Evaluation of Serum parameters in different experimental configurations

Groups	CRPMg/dl	TNFPg/mL
Control	0.10 ± 0.10	127.83 ± 8.33
ECP	0.12 ± 0.10	133.50 ± 3.51
CRG	0.60 ± 0.33 [ab]	243.50 ± 18.59 [ab]
C&E	0.37 ± 0.21 [ab]	186.00 ± 3.85 [ab]

The values are mean ± SD value, n = six in each group, [a] show the difference in statistics. With a control group, [b] statistical disparity according to illness group, (P < 0.05). ECP: Group of aqueous extract of *Eucalyptus globulus* leaves, CRG: group of Carrageenan, C&E: group of carrageenan then aqueous extract of *Eucalyptus globulus* leaves.

(P< 0.05) in contrast to the control group and a significant decrease (P< 0.05) compared to the CRG and C&E groups. In other words, the ECP reduced the count of WBC in the C&E group in contrast to the CRG group and caused a slight increase in the ECP group compared to the control group. The effect of ECP on lymphocyte count increased in the ECP group as shown in Table 1, with a significant increase (P< 0.05) in contrast to the control group and a significant decrease (P< 0.05) compared to the CRG and C&E groups. In addition, there are no significant differences. (P = 0.05) There is a significant difference (P = 0.05) in monocyte counts between the ECP and control groups. Table 2 showed a significant increase (P< 0.05) in the level of CRP in the serum of rats belonging to the CRG group compared to the all-experimental group. There are no significant differences (P = 0.05) between the ECP and control groups. These results are consistent with Study[26]. Finally, Table 2 shows a descending order of TNF levels in rats' serum among the experimental groups. The highest level (243.50 ± 18.59 Pg/mL) was recorded by Up, with highly significant differences (P< 0.05) compared to all other experimental groups, including the control group. It was followed by the treatment group that was given CRG for fifteen days, followed by the treatment period with ECP for fifteen days (186.00 ± 3.85 Pg/mL), then the ECP and the control groups (133.50 ± 3.51 Pg/mL) and (127.83 ± 8.33 Pg/mL), respectively, between which there were no notable variations (P = 0.05).

DISCUSSION

The effect of Carrageenan on the Blood parameters levels

According to experimental data in Tables 1 and 2, CRG changes the microbiota, stimulates the release of pro-inflammatory cytokines, activates innate immunity pathways, and initiates or intensifies the inflammatory response[27].

In general, WBC count increases in many inflammation conditions[28], such COVID-19[29], Parkinson's disease[30], rheumatism, lymphoid inflammation[31], liver diseases[32], and urinary tract inflammation[33], induced colitis[34]. In this study, increases of peripheral Leucocytes, that suggest because of carrageenan[24], by stimulated macrophages and the release of IL-1â that have a major part in the inflammatory reaction[35]. CRG can elicit an acute inflammatory response with polymorphonuclear neutrophils infiltration and exudate increasing in cells[36].

Carrageenan stimulated the production of cytokines/chemokines by neutrophils or macrophages[37], resulting tissue damage[38], and this leads to estimate the extent of damage caused by carrageenan[39]. Increase level of C-reactive protein (CRP) in the CRG group[40].

Moreover, CRG reported to affect the oxidative burst activity in neutrophils. The oxidative burst is a crucial process by which neutrophils eliminate ingested pathogens by the formation of reactive oxygen species (ROS)[41].

It seems that the mechanism responsible for this impact is the activation of certain signaling pathways linked to inflammation. CRG attaches to immune cells' toll-like receptors (TLRs)[42], setting off a series of events that lead to increased neutrophil synthesis and recruitment. This mechanism is necessary to create a successful immune response, but it can also result in excessive inflammation if it is not control appropriately[43]. That demonstrate of induced inflammation[44], as the best-known inflammatory marker in an oncological situation, CRP, represents an inflammatory agent[45].

In addition to the WBC, lymphocyte, monocyte, neutrophils, and CRP, the significant increase in the levels of TNF as inflammatory mediator in the CRG group it is consider another evidence of inflammation in this group[34,46]. One of the most effective medications for the management of autoimmune and chronic inflammatory diseases is a biologic that neutralizes TNF, a key cytokine in inflammatory responses[47]

Inflammatory reactions can be triggered directly through the production of inflammatory genes or indirectly through the induction of cell death[47].

Consequently, it shown that in a range of animal models of TNF-induced inflammatory diseases, genetically guided cell death may be able to reverse the inflammatory phenotype[48]. Cells do not always react to TNF by dying. In order to shield the organism from possible harm, protective brakes, also known as cell death checkpoints, often actively

inhibit TNF cytotoxicity, Although TNF can cause cell death, this reaction only happens when one of the checkpoints for cell death deactivated[49].

The effect of ECP on WBC count, Lymphocytes, monocytes and neutrophils

In this study we investigation to determine the ant-inflammatory of aqueous extract of *Eucalyptus globulus* (ECP) by the inflammatory indices. The data of this study in the Table 1, it is attributed to the presence of active substances in the ECP that have a protective role against inflammatory factors caused by CRG[50,51]. Most cases of inflammation are accompanied by a significant increase in the of WBC count[52], This is because these cells have primary functions in eliminating foreign bodies such as bacteria[53], cancer cells [54], and cell debris[55]. ECP has antibacterial[56], anti-inflammatory properties[57], therefore played a major role in reducing inflammation caused by the effect of CRG in the C&E group.

Lymphocytes and monocytes are types of WBC with Polymorphonuclear, Lymphocytes play a major role in correlating at a cellular level with the accumulation in lymphoid tissues[58]. As an illustration in Table 1, the evidence for clonal expansion in innate lymphocytes—which has largely been observed in cytotoxic natural killer (NK) cells responding to CMV infection is gathered, in response to this viral infection, NK cells in particular go through clonal growth in a manner specific to each antigen on their route to developing memory characteristics[59].

Aside from the fact that macrophages are the most prevalent immune cell type in adipose tissue, other immune cells, such as B cells, are also present and play significant roles in regulating adipose tissue inflammation. These factors explain the immune system's involvement in obesity-induced adipose tissue inflammation and the ensuing metabolic dysfunction[60].

Since it proven in this study that CRG caused inflammation in experimental animals in the CRG and C&E groups, which was evident through the increase in the inflammatory indicators mentioned above, including WBC, monocytes and neutrophils as clear in the data of the Table 1, ECP make to maintain the integrity of the cells exposed to inflammation and reduce the indicators of inflammation caused by CRG. This was evident in the decrease in the levels of these cells of all types in the C&E and ECP groups, while the rest of the levels of these cells were high in the CRG group. This confirms the presence of anti-inflammatory substances in the aqueous extract of *Eucalyptus* leaves[61,62].

CRG-induced inflammation allows for the evaluation of cell migration, the involvement of cytokines and chemical mediators, and protein leakage. While ECP did decrease the overall leukocyte count in the peripheral blood, it is worth noting that neutrophils comprised the largest proportion of leukocytes in the group treated with CRG. We can see that this ECP can lower the amounts of inflammatory substances made by immune cells because the ratio of neutrophils went down and the levels of monocytes, lymphocytes, CRP, and TNF went down in the blood of the test animals[63].

The effect of ECP on CRP

The liver produces CRP in response to inflammatory cytokines[64], CRP levels rise when the liver is exposed to oxidative stress[65], indicating systemic inflammation and linked to chronic diseases like cardiovascular disorders and autoimmune conditions[66]. Since CRG enhances levels of inflammation that in turn increase CRP levels, which is what was observed in the CRG group, ECP reduces the level of inflammation that in turn decrease CRP levels (Table 2), which is what we observed in the C&E group[67]. *Eucalyptus globulus* leaves' essential oils, particularly eucalyptol, have anti-inflammatory properties, preventing pro-inflammatory cytokine synthesis and encouraging anti-inflammatory mediator release, potentially lowering CRP levels[68]. *Eucalyptus globulus* extracts enhance antioxidant activity, reduce oxidative stress, lower inflammatory responses, and reduce CRP levels[69]. Essential oils are known to possess significant antioxidant action, which is related to phenolic groups like thymol, carvacrol, and maybe 1,8 cineole[70].

The effect of ECP on TNF

The cytokine TNF, which is generated by lymphocytes (T-cell)[71], and monocytes (macrophages)[72], plays a crucial role in immune cell control and systemic inflammation[73], triggering a range of immunological responses[74]. Although cytokines are not the only routes or substances that cause inflammation[75], TNF plays a critical role as a mediator in the start and spread of

inflammatory reactions[76]. The CRG showed higher TNF levels due to abnormally increased lymphocytes and monocytes, which are key secretors of TNF. Conversely, a decrease these cells in C&E group resulted in a decrease in TNF levels in this group (Table 2). The antioxidant property of *Eucalyptus globulus* leaves is due to the following monoterpenes[77]: á pinene, â pinene, limonene, â myrcene, sabinene and terpinolene have antioxidant properties[56].

CONCLUSION

A simple method similar to Iraqi folk medicine was used to get antioxidant and anti-inflammatory compounds from Eucalyptus globulus leaves. The study found that rats exposed to carrageenan for 30 and 15 days had much lower levels of inflammation than rats in the control group.

The study identifies parameters like WBC, lymphocytes, monocytes, neutrophils, CRP, and TNF to establish relationships between them. It recommends research on leaf extract's effects on the respiratory system, bronchi, and bronchioles.

ACKNOWLEDGEMENTS

I would like to thank the Faculty of Nursing, Faculty of pharmacology / University of Kerbala, Iraq.

Funding source

The authors received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Author's contribution

Mustafa K. Mushatet: Conceptualization, Methodology, Writing – Original Draft.; Asaad Abbas khalaf: Analysis, Writing – Review & Editing.; Doaa A. Hamad: Visualization, Supervision, Project Administration.; Thikra Abd Jary: Data Collection, Funding Acquisition, Resources, Supervision

REFERENCES

1. Adnan M. Bioactive potential of essential oil extracted from the leaves of *Eucalyptus globulus* (Myrtaceae). *J Pharmacogn Phytochem*. 2019;8(1):213-216.
2. Tian Y, Dong F, Zhou X, Yang X. Repellent, insecticidal and antimicrobial activities of leaf essential oils from three *Eucalyptus* species. *Chem Biodivers*. 2020;17(2):e1900580.
3. Her L, Kanjanasilp J, Chaiyakunapruk N, Sawangjit R. Efficacy and safety of *Eucalyptus* for relieving cough: a systematic review and meta-analysis of randomized controlled trials. *J Integr Complement Med*. 2022;28(3):218-226.
4. Nile SH, Keum YS. Chemical composition, antioxidant, anti-inflammatory and antitumor activities of *Eucalyptus globulus* Labill. 2018;56(10):734-742.
5. Lin T-C, Wang S-H, Huang C-C, Lai Y-C, Song T-Y, Tsai M-S. Anti-fatigue, antioxidation, and anti-inflammatory effects of *Eucalyptus* oil aromatherapy in swimming-exercised rats. *Chin J Physiol*. 2018;61(5):257-265.
6. Al-Snafi AE. The pharmacological and therapeutic importance of *Eucalyptus* species grown in Iraq. *IOSR J Pharm*. 2017;7(3):72-91.
7. Chandorkar N, Tambe S, Amin P, Madankar C. A systematic and comprehensive review on current understanding of the pharmacological actions, molecular mechanisms, and clinical implications of the genus *Eucalyptus*. *Phytomedicine Plus*. 2021;1(4):100089.
8. Noumi VD, Deli M, Nguimbou RM, et al. Particle Size Effects on Antioxydant and Hepatoprotective Potential of Essential Oil from *Eucalyptus camaldulensis* Leaves against Carbon Tetrachloride-Induced Hepatotoxicity in Rats. *Pharmacol Pharm*. 2022;13(8):253-272.
9. Aazza S, Lyoussi B, Megías C, et al. Anti-oxidant, anti-inflammatory and anti-proliferative activities of Moroccan commercial essential oils. *Nat Prod Commun*. 2014;9(4):1934578X1400900442.
10. González-Burgos E, Liaudanskas M, Viškelis J, Žvikas V, Janulis V, Gómez-Serranillos MP.

Antioxidant activity, neuroprotective properties and bioactive constituents analysis of varying polarity extracts from *Eucalyptus globulus* leaves. *J food drug Anal*. 2018;26(4):1293-1302.
11. Ghareeb MA, Sobeh M, El-Maadawy WH, et al. Chemical profiling of polyphenolics in *Eucalyptus globulus* and evaluation of its hepato–renal protective potential against cyclophosphamide induced toxicity in mice. *Antioxidants*. 2019;8(9):415.
12. Almeida IF, Fernandes E, Lima JLFC, et al. Oxygen and nitrogen reactive species are effectively scavenged by *Eucalyptus globulus* leaf water extract. *J Med Food*. 2009;12(1):175-183.
13. Chen L, Deng H, Cui H, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. 2018;9(6):7204.
14. Favacho HAS, Oliveira BR, Santos KC, et al. Anti-inflammatory and antinociceptive activities of Euterpe oleracea Mart., Arecaceae, oil. *Rev Bras Farmacogn*. 2011;21:105-114.
15. Abdulkhaleq LA, Assi MA, Abdullah R, Zamri-Saad M, Taufiq-Yap YH, Hezmee MNM. The crucial roles of inflammatory mediators in inflammation: A review. *Vet world*. 2018;11(5):627.
16. Morikawa K, Nonaka M, Narahara M, et al. Inhibitory effect of quercetin on carrageenan-induced inflammation in rats. *life Sci*. 2003;74(6):709-721.
17. Oladejo BO, Adeboboye CF, Adiji PI, Adebolu TT. Cytokine-mediated immunoregulatory activity of Lactobacillus species in a carrageenan-induced acute inflammatory model. *BioTechnologia*. 2023;104(1):53.
18. Yang K, Zhang L, Liao P, et al. Impact of gallic acid on gut health: Focus on the gut microbiome, immune response, and mechanisms of action. *Front Immunol*. 2020;11:580208. doi:10.3389/fimmu.2020.580208
19. Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF. The 1996 guide for the care and use of laboratory animals. *ILAR J*. 1997;38(1):41-48.
20. Shalaby SEM, El-Din MM, Abo-Donia SA, Mettwally M, Attia ZA. Toxicological affects of essential oils from *Eucalyptus globules* and clove Eugenia caryophyllus on albino rats. *Pol J Environ Stud*. 2011;20(2):429-434.
21. Sousa-Neto BP, Cunha FVM, Nunes DB, et al. Anti Inflammatory and Antioxidant Effects of the Indole Derived N Salicyloyltryptamine on Peritonitis and Joint Disability Induced by Carrageenan in Rodents. *Evidence Based Complement Altern Med*. 2022;2022(1):5524107.
22. Tkachenko AS, Kot YG, Kapustnik VA, et al. Semi-refined carrageenan promotes generation of reactive oxygen species in leukocytes of rats upon oral exposure but not in vitro. *Wiener Medizinische Wochenschrift*. 2021;171:68-78.
23. Marzocco S, Di Paola R, Serraino I, et al. Effect of methylguanidine in carrageenan-induced acute inflammation in the rats. *Eur J Pharmacol*. 2004;484(2-3):341-350.
24. Petryk N, Shevchenko O. Anti-inflammatory activity of mesenchymal stem cells in ë-carrageenan-induced chronic inflammation in rats: Reactions of the blood system, leukocyte-monocyte ratio. *Inflammation*. 2020;43(5):1893-1901.
25. Oladejo BO, Adiji PI. Effects of Lactic Acid Bacteria on the Haematological Indices of Carrageenan-Induced Acute Inflammation in Wistar Rats. *FUTA J Life Sci*. 2021;1(1):61-70.
26. Jafari F, Ramezani M, Nomani H, et al. Therapeutic effect, chemical composition, ethnobotanical profile of *Eucalyptus globulus*: A review. *Lett Org Chem*. 2021;18(6):419-452.
27. Guo J, Shang X, Chen P, Huang X. How does carrageenan cause colitis? A review. *Carbohydr Polym*. 2023;302:120374. doi:https://doi.org/10.1016/j.carbpol.2022.120374
28. Palladino M. Complete blood count alterations in COVID-19 patients: A narrative review. *Biochem medica*. 2021;31(3):0.
29. Feng X, Li S, Sun Q, et al. Immune-inflammatory parameters in COVID-19 cases: a systematic review and meta-analysis. *Front Med*. 2020;7:301.
30. Umehara T, Oka H, Nakahara A, Matsuno H, Murakami H. Differential leukocyte count is associated with clinical phenotype in Parkinson's disease. *J Neurol Sci*. 2020;409:116638.
31. Moradi EV, Teimouri A, Rezaee R, et al. Increased age, neutrophil-to-lymphocyte ratio (NLR) and white blood cells count are associated with higher COVID-19 mortality. *Am J Emerg Med*. 2021;40:11-14.
32. Costa D, Simbrunner B, Jachs M, et al. Systemic inflammation increases across distinct stages of advanced chronic liver disease and correlates with decompensation and mortality. *J Hepatol*. 2021;74(4):819-828.
33. Yang J, Qiu X, Zhou M, Wang D. Crocetin attenuating Urinary tract Infection and adherence of uropathogenic *E. coli* in NRK 52E cells via an inflammatory pathway. *J Food Biochem*. 2021;45(12):e13998.
34. Lopes AH, Silva RL, Fonseca MD, et al. Molecular basis of carrageenan-induced cytokines production in macrophages. *Cell Commun Signal*. 2020;18:1-17.

35. Piccioli P, Rubartelli A. The secretion of IL-1â and options for release. In: *Seminars in Immunology*. Vol 25. Elsevier; 2013:425-429.
36. Ye H, Sun L, Li J, et al. Sesamin attenuates carrageenan-induced lung inflammation through upregulation of A20 and TAX1BP1 in rats. *Int Immunopharmacol*. 2020;88:107009. doi:https://doi.org/10.1016/j.intimp.2020.107009
37. Liu Q, Zhao Y, Dong S, et al. Characteristics of Neutrophil Migration and Function in Acute Inflammation Induced by Zymosan and Carrageenan in the Mice Air Pouch Model. *Inflammation*. 2024:1-14.
38. Lessa TLA dos S, Correia TML, Santos TC dos, et al. A novel diselenide attenuates the carrageenan-induced inflammation by reducing neutrophil infiltration and the resulting tissue damage in mice. *Free Radic Res*. 2024;58(4):229-248.
39. Rahmouni F, Hamdaoui L, Rebai T. In vivo anti-inflammatory activity of aqueous extract of Teucrium polium against carrageenan-induced inflammation in experimental models. *Arch Physiol Biochem*. 2017;123(5):313-321.
40. Idris R, Akor-Dewu M, Abdulsamad A, Ciroma F, Shittu S-T. Omega 3 Fatty Acid and Vitamin A Ameliorate Carrageenan-induced Joint Inflammation in Wistar Rats. *ACTA Pharm Sci*. 58(3).
41. Sokolova E V, Karetin Y, Davydova VN, et al. Carrageenans effect on neutrophils alone and in combination with LPS in vitro. *J Biomed Mater Res Part A*. 2016;104(7):1603-1609.
42. Bhattacharyya S, Gill R, Chen ML, et al. Toll-like receptor 4 mediates induction of the Bcl10-NFêB-interleukin-8 inflammatory pathway by carrageenan in human intestinal epithelial cells. *J Biol Chem*. 2008;283(16):10550-10558.
43. Guo J, Han S, Lu X, et al. ê-Carrageenan hexamer have significant anti-inflammatory activity and protect RAW264. 7 Macrophages by inhibiting CD14. *J Funct Foods*. 2019;57:335-344.
44. Begum R, Sharma M, Pillai KK, Aeri V, Sheliya MA. Inhibitory effect of Careya arborea on inflammatory biomarkers in carrageenan-induced inflammation. *Pharm Biol*. 2015;53(3):437-445.
45. Gibson RJ, Bowen JM. Biomarkers of regimen-related mucosal injury. *Cancer Treat Rev*. 2011;37(6):487-493. doi:https://doi.org/10.1016/j.ctrv.2011.05.007
46. Mehrzadi S, Khalili H, Fatemi I, Malayeri A, Siahpoosh A, Goudarzi M. Zingerone mitigates carrageenan-induced inflammation through antioxidant and anti-inflammatory activities. *Inflammation*. 2021;44:186-193.
47. Van Loo G, Bertrand MJM. Death by TNF: a road to inflammation. *Nat Rev Immunol*. 2023;23(5):289-303.
48. Anderton H, Wicks IP, Silke J. Cell death in chronic inflammation: breaking the cycle to treat rheumatic disease. *Nat Rev Rheumatol*. 2020;16(9):496-513.
49. Delanghe T, Dondelinger Y, Bertrand MJM. RIPK1 kinase-dependent death: a symphony of phosphorylation events. *Trends Cell Biol*. 2020;30(3):189-200.
50. Adekomi da. Effect of essential oil of the leaves of Eucalyptus globulus on heamatological parameters of wistar rats. *African J Biochem Res*. 2012;6(4):46-49.
51. Wang D, Shi Z, Liu C, et al. *E. globulus* leaf EO exhibits anti-inflammatory effects by regulating GSDMD-mediated pyroptosis, thereby alleviating neurological impairment and neuroinflammation in experimental stroke mice. *J Ethnopharmacol*. 2024;319:117367.
52. Anyiam AF, Irondi EA, Obi E, et al. Effects of methanolic extract of Aloe vera on C-reactive protein and white blood cell count of Wistar rats with inflammation. *Avicenna J Med Biochem*. 2023;11(2):117-122.
53. Damiani E. Medical Conan Doyle-Leucocytes, Bacteria and Phagocytosis Before Metchnikoff. *Med nei Secoli J Hist Med Med Humanit*. 2023;35(3):17-32.
54. Mangini M, Ferrara MA, Zito G, et al. Cancer metabolic features allow discrimination of tumor from white blood cells by label-free multimodal optical imaging. *Front Bioeng Biotechnol*. 2023;11:1057216.
55. Zhu W, Su J. Immune functions of phagocytic blood cells in teleost. *Rev Aquac*. 2022;14(2):630-646.
56. Khaoula FS, Nour-Edddine C, Chaymae B, Mohammed RS, Abdellah Z, Faouzi E. Climatological effects on Chemical Composition, Biological Activity of Essential oil from *Eucalyptus globulus* Leaves and Seeds. *Trop J Nat Prod Res*. 2023;7(4).
57. Arooj B, Asghar S, Saleem M, et al. Anti-inflammatory mechanisms of eucalyptol rich *Eucalyptus globulus* essential oil alone and in combination with flurbiprofen. *Inflammopharmacology*. 2023;31(4):1849-1862.
58. Ford WL, Gowans JL, McCullagh PJ. The origin and function of lymphocytes. In: *The Thymus: Experimental and Clinical Studies*. Little, Brown & Company, Boston; 1966.
59. Adams NM, Grassmann S, Sun JC. Clonal expansion of innate and adaptive lymphocytes. *Nat Rev Immunol*. 2020;20(11):694-707.
60. Srikakulapu P, McNamara CA. B lymphocytes and adipose tissue inflammation. *Arterioscler*

Thromb Vasc Biol. 2020;40(5):1110-1122.
61. Salvatori ES, Morgan L V, Ferrarini S, et al. Anti Inflammatory and Antimicrobial Effects of *Eucalyptus spp*. Essential Oils: A Potential Valuable Use for an Industry Byproduct. *Evidence Based Complement Altern Med*. 2023;2023(1):2582698.
62. Göger G, Karaca N, Büyükkýlýç B, Demirci B, Demirci F. In vitro antimicrobial, antioxidant and anti-inflammatory evaluation of *Eucalyptus globulus* essential oil. *Nat Volatiles Essent Oils*. 2020;7(3):1-11.
63. de Siqueira Patriota LL, de Brito Marques Ramos D, e Silva MG, et al. Inhibition of carrageenan-induced acute inflammation in mice by the microgramma vacciniifolia frond lectin (MvFL). *Polymers (Basel)*. 2022;14(8):1609.
64. Duan Y, Pan X, Luo J, et al. Association of inflammatory cytokines with non-alcoholic fatty liver disease. *Front Immunol*. 2022;13:880298.
65. Mushatet MK, Jary TA, Khalaf AA, Alqabbany MA. Protective Effect of Prosopis farcta Fruit Aqueous Extract against Oxidative Stress Caused by Ethanol in Albino Rats. *Biomed Pharmacol J*. 2023;16(4):2521-2530. doi:10.13005/bpj/2826
66. Bulger DA, Minhas S, Asbeutah AA, et al. Chronic systemic inflammatory skin disease as a risk factor for cardiovascular disease. *Curr Probl Cardiol*. 2021;46(5):100799.
67. Borsani B, De Santis R, Perico V, et al. The role of carrageenan in inflammatory bowel diseases and allergic reactions: where do we stand? *Nutrients*. 2021;13(10):3402.
68. Èmiková N, Galovièová L, Schwarzová M, et al. Chemical composition and biological activities of *Eucalyptus globulus* essential oil. *Plants*. 2023;12(5):1076.
69. Novika RGH, Wahidah NJ, Rahmawati NY, Ansori ANM. Apium graveolens and *Eucalyptus globulus* Decrease Stress and Protect Folliculogenesis Marker on Woman Reproductive Health during COVID-19 Pandemic. *Indones J Pharm*. 2022:592-601.
70. Adenubi OT, Abolaji AO, Salihu T, Akande FA, Lawal H. Chemical composition and acaricidal activity of *Eucalyptus globulus* essential oil against the vector of tropical bovine piroplasmosis, Rhipicephalus (Boophilus) annulatus. *Exp Appl Acarol*. 2021;83:301-312.
71. Jovanovic D V, Di Battista JA, Martel-Pelletier J, et al. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-â and TNF-á, by human macrophages. *J Immunol*. 1998;160(7):3513-3521.
72. Cavaillon JM. Cytokines and macrophages. *Biomed Pharmacother*. 1994;48(10):445-453.
73. Ka³u¿na A, Olczyk P, Komosiñska-Vassev K. The role of innate and adaptive immune cells in the pathogenesis and development of the inflammatory response in ulcerative colitis. *J Clin Med*. 2022;11(2):400.
74. Laha D, Grant R, Mishra P, Nilubol N. The role of tumor necrosis factor in manipulating the immunological response of tumor microenvironment. *Front Immunol*. 2021;12:656908.
75. Licinio J, Wong M-L. Pathways and mechanisms for cytokine signaling of the central nervous system. *J Clin Invest*. 1997;100(12):2941-2947.
76. Fischer R, Maier O. Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. *Oxid Med Cell Longev*. 2015;2015(1):610813.
77. Graßmann J. Terpenoids as plant antioxidants. *Vitam Horm*. 2005;72:505-535.