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THE EFFECT OF ALLIUM SATIVUM EXTRACT ON TNF-A EXPRESSION IN THE MIDDLE EAR OF RATS WITH PSEUDOMONAS AERUGINOSA-INDUCED OTITIS MEDIA

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ABSTRACT
Otitis media (OM) encompasses a spectrum of inflammatory diseases of the middle ear, including acute otitis media (AOM), otitis media with effusion (OME), and chronic suppurative otitis media (CSOM). This study aimed to determine the effect of Allium sativum extract administration on TNF-α expression in the middle ear tissue of rat models with Pseudomonas aeruginosa-induced otitis media. This study was an experimental animal study using a post-test-only control group design. A total of 36 rats that met the inclusion and exclusion criteria were included in this study. The study was conducted at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Sumatera Utara, from February to June 2026. The study population consisted of healthy and active male Wistar rats (Rattus norvegicus), aged 2–3 months, in which otitis media was induced using Pseudomonas aeruginosa bacteria. The subjects were divided into six groups; based on Federer's formula, each group consisted of six ears. Data analysis was performed using ANOVA (for normally distributed data), the Kruskal–Wallis test (for non-normally distributed data), and post-hoc tests (to evaluate differences between groups). TNF-α expression in the middle ear tissue of the Pseudomonas aeruginosa-induced otitis media model rats showed an increase, with moderate and strong expression levels; the negative control group showed a decrease, with weak and moderate expression but no strong expression; the 70% concentration Allium sativum extract group showed a decrease (severe category); and the 80% and 90% concentration groups showed weak and moderate expression, with no severe categories observed. The administration of Allium sativum extract ear drops demonstrated the potential to reduce TNF-α expression in the middle ear tissue of rat models with Pseudomonas aeruginosa-induced otitis media at concentrations of 80% and 90%.

Keywords: allium sativum extract; otitis media; pseudomonas aeruginosa induction; rat middle ear; tumor necrosis factor expression

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INTRODUCTION

Otitis media (OM) is a general term used to describe inflammation of the middle ear. This condition consists of several important subclassifications that are distinguished based on disease duration, clinical manifestations, and the presence of fluid accumulation within the middle ear cavity. Otitis media is classified into acute otitis media (AOM), otitis media with effusion (OME), and chronic suppurative otitis media (CSOM). Although most cases resolve spontaneously without complications, OM has the potential to cause hearing loss and long-term sequelae. OM can be caused by bacteria or viruses. In upper respiratory tract infections (URTIs), microorganisms can migrate to the middle ear through the Eustachian tube, allowing colonization by pathogenic bacteria from the nasopharynx. (Aljohani Z et al., 2018; Jamal et al., 2022; Karppinen, 2019; Khaliif et al., 2024).)

Otitis media remains an important health problem because recurrent and chronic inflammation may lead to long-term complications, including persistent infection and hearing impairment. Chronic suppurative otitis media (CSOM) is frequently associated with bacterial infection, with Pseudomonas aeruginosa being one of the predominant pathogens isolated from middle ear infections. The persistence of this microorganism is related to its ability to produce several virulence factors and establish biofilm formation, which protects bacteria from host immune responses and reduces antibiotic penetration, contributing to treatment failure and recurrence. (Hernawan B et al., 2026; Taha AB., 2025)

According to the World Health Organization (WHO) data in 2021, otitis media is one of the major causes of hearing impairment, with an incidence rate of 10.85% or approximately 700 million cases annually worldwide. Otitis media is one of the most common infectious diseases in children, with the highest incidence occurring between 6 and 24 months of age. Anatomical factors, such as the shorter and more horizontal Eustachian tube in children, contribute to the higher incidence in this population. The incidence decreases in adults over 40 years of age, with the peak incidence among adults occurring in individuals younger than 30 years old, accounting for 91.3% of cases. (World Health Organization, 2021; Danishyar & Ashurst, 2025; Jin et al., 2024).

The World Health Organization (WHO) estimates that by 2023, approximately 250 million people, or 4.2% of the world's population, will have suffered from AOM. In Indonesia, the prevalence of AOM in adults ranges from 3.9% to 6.9%. Although many cases of AOM resolve naturally, approximately 30% to 40% of cases recur within three months, and another 10% may persist for up to a year. The global prevalence of CSOM is estimated at 65-330 million cases with otorrhea, of which approximately 60%, or 39-200 million, experience hearing loss. In Indonesia, an estimated 6.6 million individuals out of a total population of 220 million who suffer from CSOM experience hearing loss. A national survey conducted by the Indonesian Ministry of Health across seven provinces reported that the incidence of otitis media was 3.8%. (Waqqas et al, 2024; Nurafni et al, 2021; Kementrian Kesehatan Republik Indonesia, 2018; Simbolon et al., 2018) A study on a mouse model of otitis media induced by Pseudomonas aeruginosa bacteria (ATCC-27853) of 0.1 milliliters 1x108 Colony Forming Units (CFU) injected into the middle ear through tympanic membrane perforation, showed that there were histopathological changes in the middle ear of mice as well as a significant increase in the expression of inflammatory mediators such as the proinflammatory cytokine Tumor Necrosis Factor-α (TNF α) compared to the middle ear of mice that were not induced by the bacteria. (Sahin A et al., 2022)

The most common bacteria found in CSOM is Pseudomonas aeruginosa, accounting for 18-67%. Several clinical studies have shown significant resistance to several eardrop antibiotics, particularly chloramphenicol and ofloxacin, and strains that are unresponsive to topical ciprofloxacin have been found. These bacteria also frequently exhibit resistance to a variety of commonly used empirical antibiotics, making laboratory sensitivity testing crucial for determining effective therapy. (Krotter et al., 2025) Pseudomonas aeruginosa has the ability to form biofilms. The extracellular matrix, composed of polysaccharides, proteins, lipids, and extracellular DNA, acts as a physical barrier that limits antibiotic penetration into the biofilm. The bacterium is encased in an exopolysaccharide, protecting it from penetration by antibiotics, antibodies, the complement system, and phagocytic cells. These properties confer antibiotic resistance, increase relapse rates, and make eradication difficult. (Hernawan et al., 2026; Taha AB., 2025) The management of otitis media, particularly CSOM, often uses antibiotic ear drops because they are administered directly to the site of infection, resulting in optimal concentrations and the ability to suppress the growth of infecting microorganisms more effectively with minimal systemic effects. In practice, the use of antibiotic ear drops often does not comply with clinical guidelines. Many patients use antibiotic ear drops without a doctor's prescription or use them indiscriminately without considering the correct dosage,

duration, and medical indications. This irrational use of antibiotics can trigger bacterial resistance. (Selleck AM et al., 2021)

Over time, resistance to antibiotic ear drops due to improper use is increasing, necessitating the need for safe, effective, and non-resistance-inducing alternative treatments. Medicinal plants have long been used in traditional medicine and are now gaining attention as alternative therapies to address various global health challenges such as increasing antibiotic resistance. One such plant is garlic. (Uzun L et al., 2019) Garlic (Allium sativum), which belongs to the Alliaceae family, has long been recognized as a valuable spice and has been widely used as a traditional medicinal plant for the treatment of various diseases. The term "garlic" originates from the Celtic language, meaning sharp or pungent, referring to the characteristic aroma of this plant. Various studies have demonstrated that Allium sativum possesses a wide range of pharmacological effects, including antibacterial, antiviral, antifungal, antioxidant, and anti-inflammatory activities. These biological activities are mainly associated with its bioactive compounds, such as allicin, S-allyl cysteine (SAC), diallyl disulfide (DADS), and S-allyl mercaptocysteine (SAMC), which have been reported to exhibit antibacterial properties against various infectious pathogens, including bacteria commonly associated with ear infections, such as Pseudomonas aeruginosa and Staphylococcus aureus. (Fesseha et al., 2019) Allicin is known to inhibit the secretion of tumor necrosis factor-alpha (TNF-α), a proinflammatory cytokine involved in the pathogenesis of various infectious conditions, including otitis media. TNF-α plays a key role in amplifying the inflammatory response and tissue damage in middle ear infections. Reducing TNF-α expression may help reduce inflammation and accelerate tissue healing. (Uzun L et al., 2019; Moutia M et al., 2018) This study aimed to determine the effect of Allium sativum extract administration on TNF-α expression in the middle ear tissue of rat models with Pseudomonas aeruginosa-induced otitis media.

METHOD

This research is an experimental animal study with a post-test only control group design. carried out in the Pathology Laboratory Anatomy Faculty of Medicine, University of North Sumatra on February 2026 to June 2026 A total of 36 healthy male Wistar rats (Rattus norvegicus) aged 2–3 months that met the inclusion and exclusion criteria were included in this study. The animals were randomly divided into six experimental groups, with six ears in each group according to Federer's formula. Otitis media was induced by inoculating Pseudomonas aeruginosa into the middle ear.

After the otitis media model was established, each group received the assigned intervention, including standard treatment and different concentrations of Allium sativum extract. At the end of the treatment period, the rats were sacrificed, and middle ear tissue specimens were collected. The collected tissues were processed for histopathological preparation and immunohistochemical examination to evaluate Tumor Necrosis Factor-alpha (TNF-α) expression. Differences in TNF-α expression in each group were analyzed using ANOVA if the data were normally distributed. If the data were not normally distributed, the Kruskal-Wallis test was used. Post-hoc tests were performed to determine which groups showed significant differences. $p \leq 0.05$ is considered statistically significant. This study was conducted after obtaining ethical approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara, Medan, with approval number 168/KEPK/USU/2026.

RESULT

Goodness-of-Fitness Test (Kappa)

Reliability testing used Cohen's Kappa coefficient to evaluate the level of agreement between two independent assessors in categorizing the degree of TNF-α expression in the middle ear membrane of a rat otitis media model. Table 1 presents the frequency distribution of assessments and agreement statistics for four categories of TNF-α expression, namely negative, weak, moderate, and strong.

Table 1.
Frequency Distribution and Suitability Test of TNF-α Expression Assessment in the Middle Ear of Otitis Media Model Rats

Assessor 1	Assessor 2				Total	p*	Cohen's Kappa (κ)
	Negative	Weak	Currently	Strong			
Negative	6 (16.7)	0	0	0	6 (16.7)	< 0.001	0.903
Weak	0	5 (13.9)	0	0	5 (13.9)		
Currently	0	0	21 (58.3)	1 (2.8)	22 (61.1)		
Strong	0	0	1 (2.8)	2 (5,6)	3 (8.3)		

Inter-rater reliability analysis of TNF-α expression assessment in the middle ear membrane was performed on 36 valid samples. The results showed a high level of agreement between the two assessors, with exact agreement observed in 34 samples (94.4%) and minor discrepancies in only 2 samples (5.6%). Cohen's Kappa analysis demonstrated a κ value of 0.903 (p <0.001), indicating "almost perfect agreement" according to Landis and Koch criteria. These findings confirm excellent consistency and reliability of the immunohistochemical assessment method. Therefore, data from the first assessor were used for further inferential analysis.

Differences in TNF-α Expression After Intervention between Treatment Groups

A comparative analysis of the degree of TNF-α expression in the middle ear membrane between six treatment groups was performed using the non-parametric Kruskal-Wallis test. The results of the analysis showed a statistically significant difference in TNF-α expression between treatment groups (p <0.001). The epsilon-squared effect size (ε^2) value of 0.619 indicates that the difference in treatment had a large influence on the variation in TNF-α expression, amounting to 61.9%. This value is included in the category of very large effects, where an ε^2 value ≥0.14 is categorized as a large effect.

Table 2.
Differences in TNF-α Expression from the Middle Ear in Otitis Media Model Rats After Intervention between Treatment Groups

Group	TNF-α expression, n (%)				p*	Effect Size ε 2 (95% CI)
	Negative	Weak	Currently	Strong		
S	6 (100)	0	0	0	<0.001*	0.619
K (-)	0	0	4 (66.7)	2 (33.3)		
K (+)	0	2 (33.3)	4 (66.7)	0		
EAS 70%	0	0	5 (83.3)	1 (16.7)		
EAS 80%	0	2 (33.3)	4 (66.7)	0		
EAS 90%	0	1 (16.7)	5 (83.3)	0		

*Kruskal Wallis, aEpsilon square (ε2): 0.01 (small), 0.06 (medium), ≥0.14 (large)

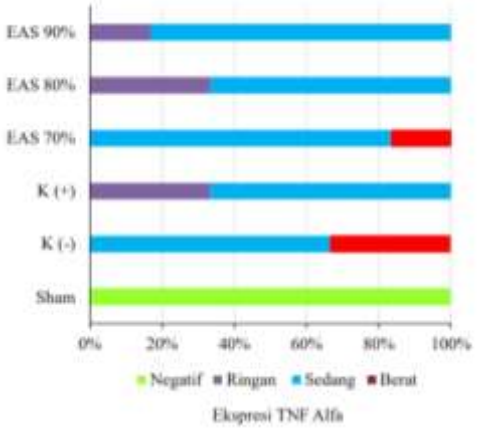

Figure 1. Differences in TNF-α Expression in the Middle Ear of Otitis Media Model Rats After EAS Intervention between Treatment Groups

Descriptively, the healthy group showed the lowest TNF-α expression (100% negative), whereas the negative control group demonstrated the highest expression, confirming successful otitis media induction by Pseudomonas aeruginosa. The positive control and Allium sativum extract groups (70%, 80%, and 90%) exhibited lower TNF-α expression than the negative control. Among the

extract groups, EAS 80% showed a distribution similar to the positive control, while EAS 70% and EAS 90% were predominantly in the moderate category. These findings indicate that Allium sativum extract reduced inflammatory responses, although TNF-α expression did not return to healthy group levels. Effect size value ($\varepsilon^2 = 0.619$) indicates that Allium sativum extract had a strong effect on changes in TNF-α expression in a mouse model of otitis media. This finding supports a post-hoc analysis to determine more specific differences between treatment groups. Therefore, after the Kruskal-Wallis test showed a significant difference, the analysis was continued with a post-hoc Dunn's test with Bonferroni correction to determine which group pairs were significantly different.

Table 3 presents the adjusted p-values and rank-biserial correlation (r) effect sizes for each intergroup comparison. This analysis was used to determine the strength and direction of differences in TNF-α expression between treatment groups.

Table 3.
Results of Dunn's Post-Hoc Test with Bonferroni Correction and Pairwise Effect Size for Comparison of TNF-α Expression in the Middle Ear of Rat Otitis Media Model

Group comparison	Adjusted p value	Rank-biserial effect size	Interpretation
Healthy vs Positive Control	0.096	0.482	Currently
Healthy vs Negative Control	<0.001	0.95	Big
Healthy vs EAS 70%	0.001	0.95	Big
Healthy vs EAS 80%	0.096	0.482	Currently
Healthy vs EAS 90%	0.025	0.647	Big
Negative Control vs Positive Control	1	0	There isn't any
Negative Control vs EAS 70%	1	0	There isn't any
Negative Control vs EAS 80%	1	0	There isn't any
Negative Control vs EAS 90%	1	0	There isn't any
Positive Control vs EAS 70%	1	0	There isn't any
Positive Control vs EAS 80%	1	0	There isn't any
Positive Control vs EAS 90%	1	0	There isn't any
EAS 70% vs EAS 80%	1	0	There isn't any
EAS 70% vs EAS 90%	1	0	There isn't any
EAS 80% vs EAS 90%	1	0	There isn't any

*Dunn's test with Bonferroni correction;
Interpretation: r,b < 0.10 (none), 0.10-0.29 (small), 0.30-0.49 (moderate), ≥0.50 (large)

Post-hoc analysis using Dunn's test with Bonferroni correction demonstrated a significant difference only between the healthy and negative control groups (adjusted $p<0.001$), with a very large effect size ($r=0.950$), indicating a marked increase in TNF-α expression following Pseudomonas aeruginosa induction. Comparisons between the healthy group and treatment groups (positive control, EAS 70%, EAS 80%, and EAS 90%) showed differences in TNF-α expression with moderate to large effect sizes; however, these differences did not reach statistical significance after Bonferroni correction. These findings suggest that Allium sativum extract treatment tended to reduce TNF-α expression toward levels observed in the healthy group.

DISCUSSION

This study was conducted to analyze the effect of Allium sativum extract on TNF-α expression in the middle ear tissue of a rat model of otitis media induced by Pseudomonas aeruginosa bacteria. TNF-α was chosen as the main parameter because it is one of the pro-inflammatory cytokines that plays an important role in acute and chronic inflammatory processes, including inflammation of the middle ear mucosa. Increased TNF-α expression indicates the activation of the local immune response to bacterial infection, while decreased expression may reflect the modulation or resolution of the inflammatory process. (Pratiwi D et al., 2022) This study involved six treatment groups, namely the healthy group, negative control, positive control that received ofloxacin, and three treatment groups that received Allium sativum extract with concentrations of 70%, 80%, and 90%.

The present study demonstrated that Pseudomonas aeruginosa induction increased TNF-α expression in middle ear tissue, confirming the development of a local inflammatory response. Treatment with ofloxacin and Allium sativum extract showed a tendency to reduce TNF-α expression compared with the negative control group, suggesting their potential role in suppressing inflammation. Although overall analysis showed significant differences among groups, post-hoc analysis revealed that not all treatment comparisons reached statistical significance. Therefore, Allium sativum extract may have potential anti-inflammatory effects on TNF-α expression; however, further studies with larger sample sizes and quantitative evaluation methods are needed to confirm its efficacy and determine the optimal concentration.

TNF-α Expression in Healthy Group and Negative Control

The healthy group in this study showed negative TNF-α expression in all samples. TNF-α expression in the middle ear tissue of healthy mice appeared very weak on IHC staining, so it was operationally categorized as negative or insignificant. This finding is consistent with the physiological condition of normal middle ear tissue that does not experience an active inflammatory process, so that the production of proinflammatory cytokines such as TNF-α is at very low basal levels. This is in line with the 2022 study by Leichtle et al., which explained that TNFα mRNA can still be assessed in healthy middle ear mucosa, although its expression is lower compared to otitis media tissue. Therefore, very weak TNF-α IHC staining in the healthy group can still be considered normal, but in terms of scoring, it can be categorized as negative or insignificant because it does not indicate significant inflammatory activation. (Leichtle et al., 2022)

In contrast to the healthy group, the negative control group showed increased TNF-α expression. In this group, TNF-α expression was primarily in the moderate and severe categories, indicating that Pseudomonas aeruginosa induction can trigger a local inflammatory response in the middle ear tissue. This is consistent with the concept of otitis media pathogenesis, where bacterial infection of the middle ear can activate an immune response that leads to the release of proinflammatory cytokines, inflammatory cell infiltration, and changes in mucosal structure. Pseudomonas aeruginosa is a gram-negative bacterium that frequently plays a role in ear infections. This bacterium possesses various virulence factors, including the ability to form biofilms, produce proteolytic enzymes, and trigger a persistent inflammatory response. Components of the gram-negative bacterial cell wall, particularly lipopolysaccharide, can activate inflammatory pathways through innate immune receptors and increase the production of proinflammatory mediators, including TNF-α.

Tumor Necrosis Factor-α plays a role in increasing vascular permeability, recruiting leukocytes to the site of inflammation, activating macrophages and neutrophils, and amplifying the release of other inflammatory mediators. In otitis media, increased TNF-α can cause damage to the middle ear mucosa, increase secretions, and maintain the inflammatory process. Therefore, the high expression of TNF-α in the negative control group indicates that the otitis media model used in this study successfully elicited a biologically relevant inflammatory condition. This finding is in line with a study by Pratiwi et al. in 2022, which reported that TNF-α contributes to the inflammatory process in active otitis media. The increase in TNFα in this condition indicates that this mediator plays a crucial role in maintaining inflammation. (Pratiwi et al., 2022)

TNF-α Expression in Ofloxacin Positive Control Group

The positive control group treated with ofloxacin showed reduced TNF-α expression compared with the negative control group, indicating a decrease in the inflammatory response in the middle ear tissue. Ofloxacin, as a fluoroquinolone antibiotic, inhibits bacterial DNA replication by targeting DNA gyrase and topoisomerase IV, leading to reduced bacterial load and subsequent suppression of pro-inflammatory mediator production, including TNF-α. However, TNF-α expression did not

return to levels observed in the healthy group, suggesting that residual inflammation may persist despite effective antibacterial therapy.

This condition may be caused by pre-existing mucosal damage, such as residual inflammatory mediators and immune responses that have not yet fully resolved, or the presence of biofilms that cause more persistent inflammation. Thus, the results in the positive control group showed that ofloxacin had an effect in reducing TNF-α expression, but did not completely eliminate the inflammatory response in the middle ear tissue during the observation period of this study. These findings align with research by Khomtchouk et al., who developed a mouse model of chronic suppurative otitis media with Pseudomonas aeruginosa inoculation. The study demonstrated that ofloxacin, as a standard fluoroquinolone therapy, only resulted in a temporary reduction in bacterial load and was not always able to permanently eradicate the infection. This supports the interpretation that TNF-α expression in the ofloxacin group may be reduced, but not completely eliminated, as the inflammatory process may persist after antibiotic therapy. (Khomtchouk KM et al., 2020)

TNF-α Expression in the 70% Allium sativum Extract Group

The group receiving 70% concentration of Allium sativum extract showed that TNF-α expression was still predominantly in the moderate category, with some samples in the severe category. This finding indicates that 70% EAS has a tendency to reduce TNF-α expression but the effect is not optimal. Active compounds in Allium sativum, such as allicin, S-allyl cysteine, diallyl disulfide, and other organosulfur compounds, are known to have antibacterial, antioxidant, and anti-inflammatory activities, but the effects of these compounds are greatly influenced by concentration, stability, tissue penetration ability, and duration of exposure to the target tissue.

The EAS 70% group showed a partial reduction in TNF-α expression, suggesting that this concentration may begin to exert an anti-inflammatory effect but was insufficient to achieve optimal suppression of inflammation. The persistent TNF-α expression may be related to the strong inflammatory response induced by Pseudomonas aeruginosa infection, including possible mucosal damage and biofilm formation. The persistent TNF-α expression observed in the EAS 70% group may be associated with the strong inflammatory response induced by Pseudomonas aeruginosa infection. As reported by Artono et al., Pseudomonas aeruginosa can form biofilms that contribute to persistent infection, increased therapeutic resistance, and prolonged inflammation. These findings suggest that 70% Allium sativum extract may provide initial anti-inflammatory effects but may not be sufficient to optimally suppress TNF-α expression. (Artono et al., 2020) These findings are consistent with Recinella et al., who reported that Allium sativum extract exhibits anti-inflammatory activity by reducing inflammatory mediators, including TNF-α, NF-κB, IL-6, and COX-2. The persistence of higher TNF-α expression in the EAS 70% group may be related to a concentration-dependent effect, suggesting that this concentration has potential therapeutic activity but may be insufficient to achieve optimal suppression of inflammation. (Recinella L et al., 2022)

TNF-α Expression in the 80% Allium sativum Extract Group

The EAS 80% group showed lower TNF-α expression compared with the EAS 70% group, with no severe expression observed. This finding suggests that 80% Allium sativum extract may provide a more effective anti-inflammatory response. The improved effect may be associated with a higher concentration of bioactive compounds, enhancing the ability to suppress inflammatory pathway activation. The anti-inflammatory effect of Allium sativum may be mediated through inhibition of the NF-κB signaling pathway. As described by Melguizo-Rodríguez et al., organosulfur compounds in garlic, including allicin, SAC, DADS, and DATS, can modulate inflammatory responses by suppressing NF-κB activation. This inhibition reduces the transcription of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6, thereby contributing to decreased TNF-α expression in tissues. (Melguizo-Rodriguez L et al., 2022) Uzun et al. reported that garlic derivatives exhibit antimicrobial activity against pathogens commonly associated with chronic external and middle ear

infections, including Pseudomonas aeruginosa. Therefore, the reduction of TNF-α expression observed in the EAS 80% group may be attributed not only to the direct anti-inflammatory effects of Allium sativum but also to its antimicrobial activity, which reduces bacterial stimulation and subsequently decreases inflammatory cell activation and pro-inflammatory cytokine production. (Uzun L et al., 2019)

TNF-α Expression in the 90% Allium sativum Extract Group

The EAS 90% group showed reduced TNF-α expression without severe inflammatory expression, indicating its potential anti-inflammatory effect in the otitis media model. However, the expression pattern was not markedly different from the EAS 80% group, suggesting a possible plateau effect in which increasing extract concentration does not always result in a proportional increase in therapeutic response. This phenomenon may be influenced by several factors, including tissue penetration, bioactive compound stability, inflammatory status, and the maximum biological response capacity of the tissue. Sun et al. explained that the bioactivity of plant extracts is influenced by many factors, including extraction method, solvent type, pH, temperature, extraction time, stability of the active compound, and bioavailability. Therefore, increasing the extract concentration does not necessarily result in a proportional increase in biological effects if there are limitations on the stability, release, or availability of the active compound in the target tissue. (Sun S el al., 2025) Thus, although 90% Allium sativum extract showed a tendency toward anti-inflammatory effects, the results of this study cannot yet conclude that 90% is the most effective concentration. Determining the optimal concentration requires further research that comprehensively assesses effectiveness, stability, safety, and local toxicity.

Comparison of Allium sativum Extract Effects between Concentrations

The EAS 80% and 90% groups demonstrated lower TNF-α expression compared with the EAS 70% group, as indicated by the absence of severe TNF-α expression. These findings suggest a potential trend between higher Allium sativum extract concentrations and greater suppression of inflammation. However, this cannot be confirmed as a definitive dose-response relationship because post-hoc analysis did not show significant differences between treatment concentrations. The lack of statistical significance may be related to the limited sample size and the conservative nature of Bonferroni correction, which may reduce the ability to detect moderate differences between groups. Therefore, Allium sativum extract, particularly at concentrations of 80% and 90%, may have potential in reducing TNF-α expression, although further studies are needed to determine the optimal effective concentration. Although antibiotics remain the primary treatment for Pseudomonas aeruginosa otitis media, Allium sativum extract may represent a potential adjunctive approach by targeting both antimicrobial activity and inflammatory regulation. However, further studies evaluating efficacy, safety, preparation stability, and ototoxicity are required before clinical application.

CONCLUSION

The administration of Allium sativum extract ear drops demonstrated the potential to reduce TNF-α expression in the middle ear tissue of Pseudomonas aeruginosa-induced otitis media rat models. The anti-inflammatory effect was more evident at concentrations of 80% and 90%, as shown by lower TNF-α expression without severe inflammatory categories compared with the negative control group. These findings indicate that Allium sativum extract may modulate the inflammatory response in otitis media through the reduction of TNF-α expression.

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