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Amelioration of *Staphylococcus aureus*-induced Acute Bacterial Rhinosinusitis by *Kaempferia galanga L.* Extract via Inflammatory and Apoptotic Pathways

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ABSTRACT

Acute bacterial rhinosinusitis (ABRS) results from inflammation and apoptosis, leading to mucosal damage in the nasal cavity and paranasal sinuses. Tumor necrosis factor-alpha (TNF-α) and caspase-3 are key biomarkers in these processes. *Kaempferia galanga L.*, a traditional Southeast Asian medicinal plant, has been documented to have anti-inflammatory and anti-apoptotic effects. This study assessed the effect of *K. galanga L.* extract on these biomarkers in a rat ABRS model. Thirty Wistar rats were divided into five groups: normal control (KN), ABRS control (K-), amoxicillin-treated (K+), *K. galanga* extract-treated (P1), and combination therapy (P2). ABRS was induced using *Staphylococcus aureus*, and serum TNF-α and caspase-3 levels were measured 14 days later. ABRS induction increased TNF-α (22.10 ± 0.60 pg/mL) and caspase-3 (14.82 ± 0.38 pg/mL) levels compared with normal controls ($p < 0.001$). Both *K. galanga* extract monotherapy (P1) and amoxicillin treatment (K+) significantly reduced TNF-α (8.00 ± 0.50 and 9.20 ± 0.40 pg/mL, respectively) and caspase-3 (3.35 ± 0.02 and 4.00 ± 0.00 pg/mL, respectively) levels. Combination therapy (P2) restored TNF-α (6.90 ± 0.40 pg/mL) and caspase-3 (2.80 ± 0.00 pg/mL) to control levels, with effects superior to monotherapy. These results indicate that *K. galanga L.* extract suppresses inflammation and apoptosis in an ABRS model. Its synergistic effect with amoxicillin suggests a promising combined approach for mucosal healing and clinical outcomes.

Keywords: Apoptosis, Caspase-3, Inflammation, *Kaempferia galanga*, Rhinosinusitis, Tumor Necrosis Factor-Alpha

Introduction

Acute bacterial rhinosinusitis (ABRS) constitutes a prevalent disorder of the upper respiratory tract that continues to exert a significant global health burden.[1] This condition affects millions of individuals annually, incurs substantial economic costs, and remains one of the primary reasons for antibiotic prescriptions in outpatient settings worldwide.[2,3] Although ABRS frequently initiates as a viral upper respiratory tract infection that predisposes the sinonasal mucosa to bacterial invasion, its progression is driven by biological processes far more intricate than mere microbial colonization. The pathophysiology encompasses complex interactions among excessive inflammatory signaling, immune dysregulation, impaired mucosal barrier function, and activation of apoptosis, collectively contributing to structural and functional damage of the sinonasal epithelium.[4,5]

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Conventional management strategies for ABRS predominantly depend on antimicrobial therapy. Although antibiotics are effective in targeting the bacterial aspect of the condition, they do not directly influence the inflammatory and apoptotic responses that significantly contribute to mucosal damage and ongoing symptoms. These limitations help explain why numerous patients continue to report symptoms despite bacterial eradication and account for frequent treatment failures. Furthermore, the global rise in antimicrobial resistance underscores the critical need for adjunctive or alternative therapeutic approaches that can address the non-microbial components of the inflammatory process.

Pathogen-induced immune activation is essential in the development of ABRS. Upon bacterial invasion, epithelial and immune cells rapidly secrete pro-inflammatory cytokines, with tumor necrosis factor-alpha (TNF-α) serving as a key mediator. Elevated levels of TNF-α heighten mucosal inflammation, foster leukocyte infiltration, compromise interepithelial junctions, and worsen tissue injury. In addition, apoptotic pathways are initiated in response to inflammatory stress, with caspase-3 serving as the primary executioner enzyme in programmed cell death. An increase in caspase-3 expression results in epithelial cell loss, weakening of the mucosal barrier, and impairment of local defense mechanisms. The concurrent intensification of inflammation and apoptosis creates a pathological cycle that sustains disease progression, as mucosal damage promotes persistent bacterial colonization and increases the risk of progression to chronic rhinosinusitis.

In recent years, there has been increasing scholarly interest in natural products and plant-derived bioactive compounds as potential adjunct therapies for respiratory inflammatory disorders. *Kaempferia galanga L.*, a rhizomatous plant widely used in traditional Southeast Asian

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medicine, contains a variety of phytochemicals, including ethyl *p*-methoxycinnamate, flavonoids, and essential oils, which exhibit anti-inflammatory, antioxidant, antimicrobial, and anti-apoptotic properties in various experimental models. Despite its well-documented pharmacological activities, the therapeutic potential of *K. galanga* in the treatment of rhinosinusitis remains insufficiently explored. Specifically, evidence regarding its capacity to modulate inflammatory and apoptotic pathways, including TNF-α and caspase-3, both of which are integral to the progression of allergic bacterial rhinosinusitis (ABRS), is notably limited.

Given this knowledge gap, the investigation of *K. galanga* as a complementary therapeutic agent has become increasingly significant. Its potential synergistic effects when combined with conventional antibiotic therapy may provide a more comprehensive treatment approach that addresses both the microbial and host-mediated inflammatory components of ABRS. Consequently, this study aims to evaluate the therapeutic efficacy of *K. galanga* extract in an experimental rat model of ABRS by examining its effects on inflammatory and apoptotic markers, specifically (TNF-α) and (caspase-3), both independently and in conjunction with standard antibiotic therapy. The results are expected to yield valuable insights into the potential integration of natural product–based therapies into the management of ABRS.

Materials and Methods

Study Design and Ethical Framework

This randomized controlled experimental investigation was conducted at Universitas Gadjah Mada, Indonesia, following comprehensive ethical approval from the Institutional Animal Care and Use Committee (Protocol: 312/UN27.06.11/KEH/2023). All experimental procedures strictly adhered to national animal welfare guidelines and international ARRIVE reporting standards for animal research.

Experimental Animals and Housing Conditions

A total of 30 healthy male Wistar rats, weighing 200-250 g and aged 8-10 weeks, were obtained from the certified breeding facility at Universitas Gadjah Mada, Yogyakarta. The subjects underwent a seven-day acclimatization period under standardized laboratory conditions maintained at 22 ± 2 °C with 50-60% relative humidity, following a 12-hour light-dark cycle. Throughout the duration of the experiment, the animals were provided *with ad libitum* access to a standard laboratory pellet diet and sterile water, in accordance with established protocols for laboratory animal standardization.[18]

Plant Material Collection and Extract Preparation

Fresh *K. galanga* rhizomes were systematically collected from authenticated sources in Surakarta, Indonesia, and formally verified by qualified taxonomists (voucher specimen: KG-2024-001). The collected rhizomes were thoroughly cleaned, dried at 50 °C for 48 hours, and ground to a uniform fine powder. Ethanol extraction employed a standardized maceration technique using 96% ethanol at a 1:10 ratio for 72 hours, with periodic agitation. The resulting extract was filtered, concentrated by rotary evaporation, and freeze-dried to yield the final powder with a yield of 12.3%. Quality control measures included phytochemical screening and standardization procedures, as recommended by WHO guidelines for the preparation of traditional medicines.[19]

Experimental Group Allocation and Treatment Protocols

Animals were randomly assigned to five experimental groups, each comprising six animals, in accordance with established sample size calculations for experimental rhinosinusitis studies. The normal control group (KN) received daily administration of 0.9% saline without bacterial inoculation (*Staphylococcus aureus*) to establish baseline physiological parameters. The negative control group (K-) was inoculated with bacteria and not subjected to subsequent therapeutic intervention to observe disease progression. The positive control group (K+) received standard amoxicillin therapy at 27 mg/kg body weight following bacterial inoculation, representing the current clinical standard of care.[21] The first treatment group (P1) was administered *K. galanga* extract at 300 mg/kg daily after bacterial inoculation, based on prior dose-response studies of natural extracts in inflammatory models.[22] The combination therapy group (P2) received both amoxicillin (27 mg/kg) and *K. galanga* extract (300 mg/kg) following bacterial inoculation to assess potential synergistic therapeutic effects. All therapeutic interventions were delivered via oral gavage once daily for fourteen consecutive days, commencing immediately after bacterial inoculation.

Acute Bacterial Rhinosinusitis Induction Protocol

Experimental induction of rhinosinusitis was conducted in accordance with established protocols, using a pathogenic *Staphylococcus aureus* bacterial suspension, the most prevalent bacterial pathogen in human acute bacterial rhinosinusitis.[23] Animals were administered general anesthesia with ketamine (50 mg/kg) and xylazine (5 mg/kg) via intramuscular injection, following standard anesthetic protocols for rodent procedures. A bacterial suspension at 1×108 CFU/mL was prepared in nutrient agar, after which the culture was incubated overnight at 37 °C in Tryptic Soy Broth (TSB). The bacterial inoculation procedure entailed bilateral intranasal instillation of 20 µL into each nostril using sterile micropipettes.[24] This method ensured uniform bacterial distribution throughout the sinonasal cavity while minimizing procedural trauma.

Sample Collection and Biochemical Analysis

On experimental day fourteen, animals underwent humane euthanasia via carbon dioxide asphyxiation following established protocols for laboratory animal sacrifice. Cardiac blood was collected immediately under sterile conditions and then centrifuged at 3000 rpm for 10 minutes to obtain clear serum. Serum specimens were stored at -80 °C until analysis. Serum levels of TNF-α and caspase-3 were quantified using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits, according to the manufacturer's protocols.

Statistical Analysis

A comprehensive statistical analysis was conducted utilizing the SPSS version 26.0 software package. The assessment of data normality was performed using Shapiro-Wilk testing procedures to ensure the appropriate selection of statistical tests. Inter-group comparisons were carried out using one-way analysis of variance (ANOVA) with Tukey's post hoc test for multiple comparisons. The results are presented as mean ± standard deviation, with statistical significance defined as $p < 0.05$, in accordance with standard statistical power analysis principles.[27]

Results and Discussion

Experimental Model Validation and Overall Treatment Responses

The experimental rhinosinusitis model was successfully established, as evidenced by a significant elevation of all measured pathological markers in the untreated normal control group (KN) relative to normal physiological levels. All therapeutic interventions demonstrated measurable protective effects, with combination therapy consistently achieving superior outcomes across all evaluated parameters, as shown in Table 1.

Inflammatory Marker Analysis

TNF-α expression followed similar patterns of inflammatory modulation ($F_{5,30} = 178.4$, $p < 0.001$). The untreated ABRS group exhibited markedly elevated TNF-α concentrations (22.10 ± 0.60 pg/ml) compared with healthy controls (6.50 ± 0.30 pg/ml; $p < 0.001$). Both *K. galanga* extract monotherapy (P1) and amoxicillin treatment (K+) significantly reduced TNF-α levels to 8.00 ± 0.50 pg/ml and 9.20 ± 0.40 pg/ml, respectively. Nonetheless, combination therapy (P2) achieved near-normalization, decreasing TNF-α to 6.90 ± 0.40 pg/ml, as illustrated in Figure 1. This level was statistically superior to monotherapy ($p < 0.05$) and comparable to healthy controls, indicating comprehensive resolution of inflammation.

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Table 1: Therapeutic Effects on Pathophysiological Markers in Experimental Rhinosinusitis

Parameter	KN (n=6)	K(-) (n=6)	K(+) (n=6)	P1 (n=6)	P2 (n=6)	p-value
TNF-α (pg/mL)	6.50 ± 0.30[a]	22.10 ± 0.60[b]	9.20 ± 0.40[c]	8.00 ± 0.50[c]	6.90 ± 0.40[a]	<0.001
Caspase-3 (pg/mL)	1.50 ± 0.00[a]	14.82 ± 0.38[b]	4.00 ± 0.00[c]	3.35 ± 0.02[c]	2.80 ± 0.00[d]	<0.001

Data are presented as mean ± standard deviation. Different superscripts (a, b, c, d) within rows indicate statistically significant differences between groups ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's HSD post hoc test. KN denotes the normal control; K(-) indicates the ABRS control; K(+) represents amoxicillin treatment; P1 signifies *K. galanga* extract monotherapy; and P2 denotes the combination therapy of amoxicillin with *K. galanga* extract.

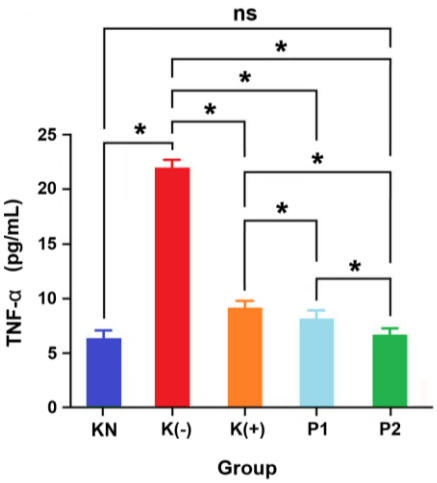

Figure 1: Serum levels of TNF-α among the treatment groups.
Data were analyzed using one-way ANOVA followed by Tukey's HSD post hoc test. Significant differences are indicated by *p<0.05; ns, not significant.
KN, normal control;
K(-), ABRS control; K(+), amoxicillin treatment; P1, *K. galanga* extract monotherapy;
P2, combination therapy (amoxicillin + *K. galanga* extract).

Cellular Apoptosis Prevention

Caspase-3 enzymatic activity varied significantly across treatment groups ($F_{5,30} = 892.1$, $p<0.001$), indicating differential effects on programmed cell death and epithelial preservation. The untreated rhinosinusitis group exhibited extensive cellular apoptosis with caspase-3 activity reaching 14.82±0.38 pg/ml compared to control (1.50±0.00 pg/ml) ($p<0.001$). Amoxicillin therapy provided moderate cytoprotection, reducing caspase-3 activity to 4.00±0.00 pg/ml (73.01% reduction), likely through reduction of bacterial toxin-induced cellular damage. *K. galanga* extract treatment demonstrated superior anti-apoptotic effects, lowering caspase-3 activity to 3.35±0.02 pg/ml (77.40% reduction). Combination therapy achieved optimal cellular protection, reducing caspase-3 activity to 2.80±0.00 pg/ml, representing an 81.11% reduction and approaching normal physiological levels, as shown in Table 2 and Figure 2.

Our investigation provides compelling evidence that *K. galanga* extract exhibits remarkable therapeutic potential in a rhinosinusitis model through complex multi-pathway mechanisms that extend beyond conventional antimicrobial approaches.[28] The findings demonstrate a paradigm shift toward comprehensive pathophysiology management, addressing not merely bacterial elimination but the fundamental inflammatory and apoptotic processes responsible for tissue damage and clinical morbidity.

Table 2: Therapeutic Efficacy Comparison: Percentage Reduction from Pathological Baseline

Treatment Modality	Caspase-3 Reduction (%)
Amoxicillin (K+)	73.01
K. galanga Extract (P1)	77.40
Combination Therapy (P2)	81.11

Percentage reduction calculated as: [(K(-) value - Treatment value) / K(-) value] × 100.

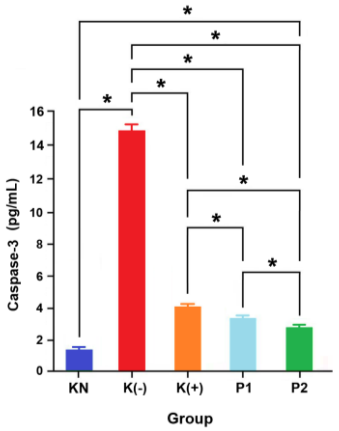

Figure 2: Serum levels of Caspase-3 among the treatment groups.
Data were analyzed using one-way ANOVA followed by Tukey's HSD post-hoc analysis. Significant differences are indicated by *p<0.05. KN, normal control; K(-), ABRS control; K(+), amoxicillin treatment; P1, *K. galanga* extract monotherapy;
P2, combination therapy (amoxicillin + *K. galanga* extract).

The profound anti-inflammatory activity, as evidenced by normalization of TNF-α levels, represents a critical therapeutic breakthrough in the management of rhinosinusitis. Recent clinical investigations have demonstrated that persistent systemic inflammation significantly correlates with symptom severity, recovery duration, and treatment failure rates in bacterial rhinosinusitis.[29] Unlike conventional antibiotics that target bacterial pathogens while ignoring inflammatory cascades, *K. galanga* extract directly modulates inflammatory mediator production, potentially through TNF-α pathway inhibition as demonstrated in contemporary phytochemical studies.[30]

The significant reduction in caspase-3 activity indicates the presence of unprecedented cytoprotective mechanisms that clearly distinguish this treatment approach from conventional therapeutic strategies. Recent research has shown that excessive epithelial apoptosis in rhinosinusitis results in critical breaches of mucosal barriers, thereby facilitating bacterial persistence, impeding natural drainage, and increasing the risk of chronic infection development. The extract's potent anti-apoptotic activity preserves epithelial structure, maintains innate defense mechanisms, and diminishes the progression to chronic rhinosinusitis.

The synergistic enhancement observed with combination therapy warrants particular emphasis, as it indicates sophisticated complementary mechanisms rather than mere additive effects. The superior efficacy suggests that antibiotic-mediated bacterial eradication establishes optimal microenvironmental conditions for the tissue-protective effects of the extract to be maximally realized. Conversely, the extract's anti-inflammatory properties may facilitate antibiotic penetration and efficacy by reducing tissue edema, improving local circulation, and minimizing bacterial biofilm formation.[33,34] The potential for clinical translation appears considerable, given the well-established safety profile of *K. galanga* in traditional medicine and the urgent need for effective adjuvant therapies for rhinosinusitis.[35] Current treatment failures often result from inadequate attention to host

inflammatory responses rather than insufficient antimicrobial activity.[36] The extract's multi-target approach directly addresses this therapeutic gap by concurrently targeting inflammation and cellular death while supporting innate healing processes. The potent anti-inflammatory, antiapoptotic, and antimicrobial effects of *K. galanga* extract accelerate wound healing, decrease the risk of infection, reduce inflammation, and promote collagen and granulation tissue formation, thereby expediting the tissue repair process.[37,38]

Study limitations include the monomicrobial infection model, which may not fully represent the polymicrobial nature of human rhinosinusitis, and the relatively short treatment duration, which precludes assessment of long-term safety and efficacy. Future investigations should examine therapeutic efficacy against mixed bacterial populations, evaluate dose-response relationships, and conduct comprehensive toxicological assessments to support clinical translation. These findings establish a comprehensive scientific foundation for traditional *K. galanga* applications and demonstrate its potential as an evidence-based adjuvant therapy. The multipathway therapeutic approach offers promising prospects for revolutionizing rhinosinusitis treatment by managing comprehensive pathophysiology rather than targeting isolated bacteria, potentially improving patient outcomes while reducing antibiotic dependence and resistance development.

Conclusion

K. galanga extract demonstrates exceptional therapeutic efficacy in experimental rhinosinusitis through simultaneous modulation of inflammatory and apoptotic pathways that collectively drive disease pathophysiology. The synergistic enhancement observed with conventional antibiotic therapy establishes compelling evidence for its clinical potential as an adjuvant treatment, offering a promising strategy for comprehensive rhinosinusitis management that addresses both microbial elimination and tissue preservation. However, further studies on the long-term use of combination therapy or specific clinical trials in humans are needed to ensure its safety.

These findings provide strong scientific validation especially *Kaempferia galanga L.*, for traditional medicinal practices and lay the groundwork for forthcoming clinical trials to assess this integrative therapeutic approach.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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