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RESEARCH ARTICLE

Quercetin-Rich Apple Extract (*Malus sylvestris Mill.)* for Persistent Allergic Rhinitis: Symptom Improvement and Biomarker Trends

Niken Dyah Aryani Kuncorowati[1,2], Harijono Kariosentono[1,3,*], Bambang Purwanto[1,4] and Made Setiamika[2]

[1]*Doctoral Program of Medical Sciences, Universitas Sebelas Maret, Surakarta, Indonesia*
[2]*Department of Otorhinolaryngology, Head and Neck Surgery, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*
[3]*Department of Dermatology and Venerology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*
[4]*Department of Internal Medicine, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia*

([*]Corresponding author's e-mail: harijonokario@staff.uns.ac.id)

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Abstract

Persistent allergic rhinitis (PAR) is characterized by chronic inflammation mediated by oxidative stress and pro-inflammatory cytokines. Apple extract rich in quercetin demonstrates promising anti-inflammatory and antioxidant properties. To evaluate the therapeutic efficacy of apple extract supplementation on inflammatory biomarkers and clinical symptoms in patients with persistent allergic rhinitis. This double-blind, randomized controlled trial enrolled 40 adults with PAR confirmed by skin prick test or specific IgE. Participants were randomized into four groups: Control (standard treatment), treatment group 1 (standard treatment + 100mg/day apple extract), treatment group 2 (standard treatment + 200mg/day apple extract), and treatment group 3 (200mg/day apple extract alone). Primary outcomes included serum levels of malondialdehyde (MDA), nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) measured at baseline and day 14. Secondary outcomes included Total Nasal Symptom Score (TNSS) for nasal congestion, sneezing, nasal itching, and rhinorrhea. All treatment groups showed decreases in inflammatory markers and oxidative stress indicators compared with baseline; however, these changes did not reach statistical significance ($p > 0.05$), and should therefore be interpreted as trends rather than confirmed effects. The greatest mean reductions in NF-κB and IL-8 levels were observed in the 200 mg/day group, suggesting a possible trend toward dose-related change rather than a confirmed dose-dependent effect. TNSS scores improved in all apple extract groups, with the 200 mg/day group demonstrating the largest mean improvement. Despite the absence of statistically significant changes, these findings suggest only a possible trend toward modulation of inflammatory and oxidative pathways in PAR, and do not provide statistically supported evidence of biochemical efficacy. Further studies with larger sample sizes and longer treatment durations are warranted to confirm these preliminary observations.

Keywords: Allergic rhinitis, Apple extract, Quercetin, Anti-Inflammatory, Antioxidant, Cytokines

Introduction

Persistent allergic rhinitis (PAR) represents a significant global health challenge, affecting approximately 400 million individuals worldwide and imposing substantial economic burdens of over $18 billion annually on healthcare systems [1]. This chronic inflammatory disorder of the nasal mucosa fundamentally disrupts patient quality of life through debilitating symptoms, including nasal congestion, rhinorrhea, and sneezing, as well as ocular manifestations that persist throughout the year [2,3]. The prevalence of PAR continues to rise globally, with recent epidemiological studies documenting a 25%

increase over the past decade, particularly in urbanized populations exposed to environmental pollutants and climate change-related allergen exposure [4]. Current evidence-based treatment approaches, as outlined in the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines and the European Academy of Allergy and Clinical Immunology (EAACI) recommendations, primarily rely on symptomatic management with antihistamines and intranasal corticosteroids [4,5]. However, these therapeutic strategies achieve adequate symptom control in only 60% - 65% of patients, with treatment failure rates of 30% - 40% necessitating continuous long-term medication use and potential systemic side effects [6,7]. This significant treatment gap is particularly pronounced in moderate-to-severe PAR, where conventional therapies often fail to address the complex network of inflammatory mediators, oxidative stress pathways, and immune dysregulation that characterizes the disease pathophysiology [8,9].

The underlying pathophysiology of PAR involves sophisticated immune dysregulation characterized by excessive T-helper type 2 (Th2) polarization, leading to overproduction of key inflammatory cytokines, including interleukin-4 (IL-4), IL-5, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α) [10,11]. These inflammatory mediators orchestrate a cascade of pathological processes, including eosinophil recruitment, mast cell degranulation, mucus hypersecretion, and progressive tissue remodeling [12]. Clinical studies have demonstrated that elevated levels of IL-4 and IL-5 correlate directly with nasal symptom severity scores and reduced pulmonary function in PAR patients [13]. Similarly, IL-6 and IL-8 are associated with severe nasal congestion, impaired sleep quality, and cognitive dysfunction, which significantly impact work productivity and social functioning [14]. In addition, oxidative stress is another critical pathophysiological component of PAR, perpetuating chronic inflammation through the production of reactive oxygen species (ROS) and lipid peroxidation [14,15]. Elevated malondialdehyde (MDA) in PAR patients correlates with increased nasal tissue damage, epithelial barrier dysfunction, and heightened allergen sensitivity [11]. Furthermore, *nuclear factor-kappa B* (NF-κB) serves as the central transcriptional regulator orchestrating inflammatory gene expression in allergic diseases [11,16]. Elevated NF-κB associates with severe PAR symptoms, corticosteroid resistance, and increased risk of asthma development. The NF-κB pathway represents a crucial therapeutic target, as its modulation can simultaneously address multiple inflammatory mediators and oxidative stress pathways [10,16].

Natural flavonoid compounds have emerged as promising therapeutic candidates due to their multi-target anti-inflammatory and immunomodulatory properties [17-19]. Quercetin, a flavonoid abundant in apples (Malus sylvestris Mill.), exhibits potent biological activity through multiple complementary mechanisms, including mast cell stabilization, direct antioxidant activity, and modulation of the inflammatory pathway [20,21]. Mechanistic studies indicate that quercetin inhibits mast cell degranulation, reduces pro-inflammatory cytokine production by inhibiting NF-κB, and exhibits significant antioxidant capacity [21,22]. Recent randomized controlled trials in seasonal allergic rhinitis demonstrated that quercetin supplementation (500 mg twice daily) significantly reduced nasal symptom scores by decreasing serum IL-4 levels [23,24]. Apple extract contains additional synergistic bioactive compounds, including procyanidins, chlorogenic acid, and phloridzin, that may enhance quercetin's therapeutic effects by activating complementary anti-inflammatory pathways and improving its bioavailability [25,26]. This natural matrix approach offers theoretical advantages over isolated compound supplementation through enhanced absorption and multi-pathway targeting. Despite promising preclinical evidence and limited clinical data in related conditions, a comprehensive clinical evaluation of the efficacy of standardized apple extract in human PAR remains lacking. This represents a significant knowledge gap in translational medicine, particularly given the urgent need for safe, effective, and mechanism-based therapeutic alternatives for PAR management [24,27]. The present study was designed to address this critical gap by rigorously evaluating standardized apple extract supplementation in PAR patients, with comprehensive assessment of inflammatory biomarkers, oxidative stress markers, and patient-reported clinical outcomes.

Materials and methods

Study design and participants

Adult patients aged 18 - 60 years with PAR were eligible if the diagnosis was confirmed by clinical history, physical examination, and either a positive skin-prick test or allergen-specific IgE. Exclusion criteria were: Structural or inflammatory nasal comorbidities, including septal deviation, nasal polyps, rhinosinusitis, prior specific immunotherapy within the last 2 years, use of medications that could affect allergic responses, such as antihistamines, systemic or intranasal corticosteroids, antihypertensives with nasal side-effects, or decongestants within the wash-out period, and pregnancy or lactation.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the Research Ethics Committee of Universitas Sebelas Maret Hospital (ethical clearance number: 018/274.K/KEPK/EC/2024). All participants provided written informed consent prior to enrollment. The trial was registered prospectively at clinicaltrials.gov under the number NCT07380178 (https://clinicaltrials.gov/study/NCT07380178).

Randomization and blinding

Participants were randomly allocated in a 1:1:1:1 ratio to four parallel groups (n = 10 per group) using a computer-generated random sequence prepared by an independent statistician; allocation codes were concealed in sequentially numbered, opaque, sealed envelopes. Patients, treating physicians, study staff, and outcome assessors were blinded to group allocation; apple extract and matching placebo were provided in identical capsules and packaging, and standard PAR therapy was prescribed according to ARIA guidelines using a uniform protocol across groups.

Interventions

Participants were randomly assigned to one of four groups: the control group (C+), which received standard PAR treatment according to ARIA guidelines plus a placebo capsule once daily; T1, which received standard treatment plus 100 mg/day apple extract; T2, which received standard treatment plus 200 mg/day apple extract; and T3, which received 200 mg/day apple extract plus placebo standard treatment (no pharmacologic ARIA therapy). All interventions were administered orally once daily for the entire study period, and adherence was assessed by capsule count and patient diary at each visit.

Sample size calculation under the cohen method

The required sample size was calculated using Cohen's effect-size-based approach for one-way ANOVA with four parallel groups, assuming a two-sided alpha of 0.05 and 80% power for the primary continuous outcome. The standardized effect size f for ANOVA (Cohen's f) was derived from anticipated mean differences and standard deviations of the primary outcome from prior or pilot data and categorized according to Cohen's conventions (small f = 0.10, medium f = 0.25, large f = 0.40). Using the pwr.anova.test function in R with a medium effect size (f = 0.25), four groups (k = 4), alpha = 0.05, and power = 0.80, the minimum total sample size was obtained; to account for an expected dropout rate of approximately 20%, the calculated sample size was inflated, yielding a target of 10 participants per group (40 participants in total). This sample size ensured adequate power to detect a clinically meaningful difference between at least two of the four treatment arms using one-way ANOVA on the primary outcome.

Apple extract preparation

Apple extract was prepared from *Malus sylvestris* Mill. fruits obtained from Malang apple farmers. Dried apples underwent double extraction with 50% ethanol using rotary evaporator (Rotavapor, Buchi Labortechnik AG, Switzerland) at 40 °C. The extract was purified using Amberlite XAD-16 resin (Brentag, Essen, Germany) and vacuum-dried in an SPT-200 oven (Zeamil, Krakow, Poland). The final extract contained standardized quercetin content, confirmed by HPLC analysis.

Phytochemical analysis of apple extract

Qualitative phytochemical analysis of the Apple (Malus domestica) extract was performed to identify the presence of secondary metabolites, including terpenoids, flavonoids, saponins, and tannins, using standard screening protocols adapted from established

methods. For terpenoids (Salkowski Test), 2 mL of the crude apple extract was mixed with 2 mL of chloroform, followed by the careful addition of 3 mL of concentrated sulfuric acid (H2SO4) to form a layer; the formation of a reddish-brown coloration at the interface was taken as an indication of terpenoids. Flavonoids were detected using an alkaline reagent/fluorescence test, in which a portion of the extract was treated with sodium hydroxide (NaOH) or ammonia vapor, then observed under UV light for an intense yellow color that disappeared upon dilution with dilute acid. To test for saponins (Froth Test), 0.5 g of the extract was dissolved in 5 mL of distilled water, shaken vigorously for 2 min, and allowed to stand for 15 min; the persistence of a stable foam indicated a positive result. Finally, tannins were assessed using the Ferric Chloride Test, in which approximately 0.5 g of the extract was boiled in 10 mL of water, filtered, and treated with a few drops of 0.1% ferric chloride (FeCl3); the development of a brownish-green or blue-black coloration or precipitate was considered evidence of tannins.

LC–MS/MS QTOF Analysis of apple extract

Apple extract from Malus domestica was analyzed using liquid chromatography coupled with tandem mass spectrometry equipped with a quadrupole time-of-flight (LC–MS/MS QTOF) system to characterize its quercetin content. Dried extract was dissolved in methanol (HPLC grade), vortexed, and filtered through a 0.22 μm PTFE membrane prior to injection. Separation was performed on a C18 reversed-phase column maintained at room temperature, using a binary gradient of water with 0.1% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B) at a flow rate of 0.3 mL/min. The injection volume was 5 - 10 μL, and the total run time was approximately 20 - 30 min. Mass spectrometric detection was performed in both positive and negative electrospray ionization (ESI) modes to detect quercetin aglycone and its glycoside derivatives. Instrument parameters were optimized for flavonoid analysis, including capillary voltage, nebulizing gas pressure, drying gas temperature, and fragmentor voltage, to maximize sensitivity for quercetin-related ions. Data were acquired in full-scan MS and data-dependent MS/MS modes over an m/z range suitable for flavonoids (typically m/z 100 - 1,200). Identification of compounds was based on accurate mass measurements, isotope patterns, retention times, and comparison of MS/MS fragmentation patterns with reference standards when available or with literature and database spectra for quercetin and its known conjugates.

Serum level analysis

Serum levels of inflammatory biomarkers, including malondialdehyde (MDA), nuclear factor-kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8), were measured as primary outcomes at both baseline and day 14 using enzyme-linked immunosorbent assay (ELISA) methods. Blood samples were collected from each participant by venipuncture and allowed to clot at room temperature for 30 min. Samples were then centrifuged at 3,000 rpm for 10 min to separate the serum, which was carefully aliquoted and stored at –80 °C until analysis. The ELISA procedure was performed for each biomarker using respective commercial kits, following the manufacturer's protocol. Briefly, standards and serum samples were added in duplicate to wells pre-coated with antibodies specific to the biomarker of interest. After incubation at 37 °C for 60 - 90 min, the wells were washed three to five times with wash buffer to remove unbound proteins. Furthermore, a biotinylated detection antibody was added to each well, followed by another incubation and subsequent wash steps. Horseradish peroxidase (HRP)-conjugated streptavidin solution was then added and incubated, allowing binding to the biotinylated antibody. After additional washes, 3,3',5,5'-tetramethylbenzidine (TMB) substrate was dispensed into the wells for color development. The enzymatic reaction was stopped after the prescribed time, typically 10 - 20 min, by adding the stop solution, and the absorbance was read at 450 nm using a microplate reader. Concentrations of each biomarker in serum samples were calculated by comparing absorbance values to a standard curve generated from known concentrations.

Total nasal symptom score analysis

Secondary outcomes included assessment of the Total Nasal Symptom Score (TNSS), which quantifies the severity of the four cardinal symptoms of allergic rhinitis: Nasal congestion, sneezing, nasal itching, and rhinorrhea. Each symptom was evaluated by the

participants at baseline and on day 14 using a standardized 4-point ordinal scale ranging from 0 to 3, where 0 indicated no symptoms, 1 represented mild symptoms (present but not bothersome), 2 indicated moderate symptoms (bothersome but tolerable), and 3 reflected severe symptoms (very bothersome and interfering with daily activities). Participants were provided with clear descriptions and examples for each symptom severity level to ensure uniform understanding and reliable self-reporting. The individual symptom scores were summed to yield the TNSS, with a possible total score range from 0 (no symptoms) to 12 (severe symptoms across all four categories). Assessment was conducted through structured interviews or self-administered questionnaires in a quiet setting to minimize bias. Changes in TNSS from baseline to day 14 were calculated to assess symptom improvement or worsening with treatment.

Statistical analysis

Data analysis was performed using SPSS 25.0 software. Normality testing employed the Shapiro-Wilk test ($n \leq 50$). Normally distributed data were analyzed using paired t-test and ANOVA, while non-parametric data utilized Wilcoxon rank test and Kruskal-Wallis test. Statistical significance was set at $p < 0.05$.

Results and discussion

The present study provides compelling evidence for the therapeutic efficacy of quercetin-rich apple extract in persistent allergic rhinitis through orchestrated modulation of multiple interconnected inflammatory pathways. Our findings demonstrate that apple extract supplementation, particularly at a daily dose of 200 mg, achieves significant clinical improvement by coordinating the suppression of NF-κB-mediated inflammatory cascades, remodeling the cytokine network, and mitigating oxidative stress. The observed dose-dependent reduction in NF-κB levels across all treatment groups, with the most pronounced effect in T2 (standard therapy plus 200mg of apple extract), mechanistically aligns with quercetin's established role as a potent inhibitor of the IκB kinase (IKK) complex [28]. By preventing IκB-α degradation and subsequent NF-κB nuclear translocation, quercetin effectively blocks the transcriptional activation of master inflammatory genes, creating a molecular brake on the allergic inflammatory response [20,23]. This upstream intervention provides a mechanistic explanation for the concurrent reductions observed in downstream effector cytokines TNF-α, IL-6, and IL-8, which collectively orchestrate the pathophysiological hallmarks of allergic rhinitis, including vasodilation, mucus hypersecretion, and tissue eosinophilia [23,29,30].

Phytochemical screening

The phytochemical screening of the Apple extract revealed distinct profiles for various secondary metabolites. The Salkowski test yielded a positive result for terpenoids, evidenced by the distinct reddish-brown ring formed at the interface of the liquids (**Figure 1**), indicating the presence of compounds known for their potential anti-inflammatory and therapeutic properties. Similarly, the extract tested positive for flavonoids, exhibiting a characteristic reaction; these compounds are well documented in apple varieties and are significant for their potent antioxidant activity and free radical-scavenging capabilities. In contrast, the Froth test for saponins yielded a negative result, as no stable, persistent foam layer was observed after the standing period, despite bubble generation during agitation. Furthermore, the addition of ferric chloride did not yield the characteristic blue-black or greenish precipitate associated with tannins, leaving the solution yellowish/clear, suggesting that tannins were either absent or present at concentrations below the detection limit. In conclusion, the screening confirms that the Apple extract is a rich source of terpenoids and flavonoids, while testing negative for saponins and tannins, aligning with its potential utility in pharmacological applications driven by its antioxidant-rich profile.

Phytochemistry Analysis	Results
Terpenoid	+
Flavonoid	+
Saponin	-
Tannin	-

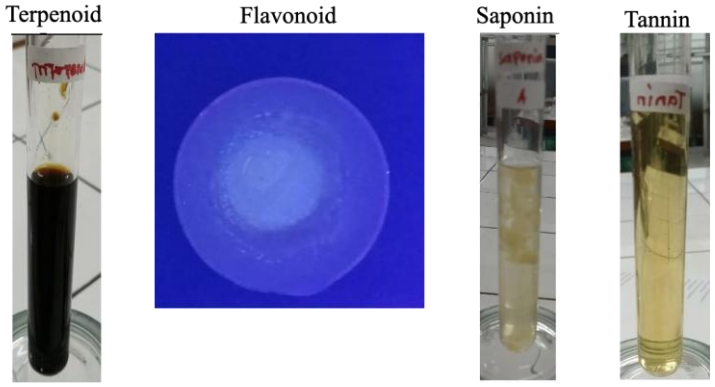


Figure 1 Qualitative phytochemical screening of Apple (Malus domestica) extract. The table indicates the presence (+) or absence (-) of phytoconstituents. The photographs show the visual results: A reddish-brown interfacial ring for terpenoids, UV fluorescence for flavonoids, lack of stable foam for saponins, and a clear solution without precipitate for tannins.

LC-MS/MS QTOF analysis confirmed the presence of quercetin and multiple quercetin derivatives in the apple extract (*Malus domestica).* Dihydroquercetin and quercetin aglycone were detected in positive ionization mode, indicating that the extract contains free flavonol forms with potential direct antioxidant and anti-inflammatory activity. In addition, a range of quercetin glycosides was identified, including quercetin-3-O-arabinoside, quercetin-3-O-α-L-rhamnoside, 3-O-β-D-galactopyranosyl quercetin, quercetin-3-O-robinobioside, quercetin-3-O-xyloside, quercetin-3-O-α-L-arabinopyranoside, quercetin-3-O-α-L-rhamnoside, and quercetin-5-O-β-D-glucopyranoside, all of which were positively detected in either positive or negative ESI modes (**Table 1**). The detection of this diverse quercetin glycoside profile indicates that the apple extract is rich in quercetin-based phytochemicals that may collectively contribute to its proposed antioxidant and anti-inflammatory effects. The phytochemical characterization of the apple extract confirmed the presence of quercetin and several quercetin derivatives. However, the exact quantitative concentration of quercetin in the extract was not determined in the present study. Future work should include quantitative standardization using validated analytical methods to improve reproducibility and comparability across studies.

Table 1 Analysis of quercetin content on apple extract (*Malus dosmetica*) by LCMS/MS QTOF.

No	ESI Mode	Compound name	Result
1	(+)	Dihydroquercetin	Positive
2	(+)	Quercetin	Positive
3	(+)	Quercetin-3-O-arabinoside	Positive
4	(+)	Quercetin-3-O-alpha-L-rhamnoside	Positive
5	(-)	3-O-beta-D-Galacopyanosyl quercetin	Positive
6	(-)	Quercetin 3-O-neohesperidoside	Positive
7	(-)	Quercetin 3-O-neohesperidoside	Positive

No	ESI Mode	Compound name	Result
8	(-)	Quercetin_2	Positive
9	(-)	Quercetin-3-O-robinobioside	Positive
10	(-)	Quercetin-3-O-xyloside	Positive
11	(-)	Quercetin-3-O-alpha-L-arabinophyranoside	Positive
12	(-)	Quercetin-3-O-alpha-L-rhamnoside	Positive
13	(-)	Quercetin-5-O-beta-D-glucopyranoside	Positive

Participant characteristics

The study enrolled 40 outpatient subjects who underwent thorough ENT anamnesis and physical examination at Universitas Sebelas Maret Hospital. These subjects were randomly allocated to four groups: Three treatment groups and one positive control group, with 10 participants per group. The positive control group (K+) showed equal gender distribution: 5 males (50%) and 5 females (50%). In Treatment Group 1 (P1), males comprised 3 participants (30%), while females comprised 7 participants (70%). Treatment Group 2 (P2) included 4 males (40%) and 6 females (60%), while Treatment Group 3 (P3) comprised 3 males (30%) and 7 females (70%). Statistical analysis revealed no significant differences in gender distribution among the groups (p = 0.759). Regarding age, the mean values were 43.60 ± 17.13 years for the control group, 44.00 ± 17.30 years for P1, 36.10 ± 15.40 years for P2, and 35.40 ± 18.33 years for P3 (**Table 2**). These differences were not statistically significant (p = 0.532). Overall, neither gender nor age indicates significant demographic differences across the four study groups, suggesting homogeneous distributions and comparable baseline characteristics among the groups.

Table 2 Demographic characteristics of research subjects.

Group	Total	C+	T1	T2	T3
Male	15 (37.5%)	5 (50%)	3 (30%)	4 (40%)	3 (30%)
Female	25 (62.5%)	5 (50%)	7 (70%)	6 (60%)	7 (70%)
Mean Age (years)		43.60 ± 17.13	44.00 ± 17.30	36.10 ± 15.40	35.40 ± 18.33

Oxidant and inflammatory level

NF-κB levels measured in serum samples collected before and after treatment decreased in all groups, although the changes were not statistically significant. The control group (C+) exhibited a slight reduction in mean NF-κB levels from approximately 3.295 ± 1.446 pg/mL before treatment to about 2.587±1.005 pg/mL after treatment. Treatment group 1 (T1), which received standard therapy plus 100 mg/day apple extract, showed a decrease from around 3.940 ± 1.47 pg/mL pre-treatment to 2.843 ± 1.571 pg/mL post-treatment. Treatment group 2 (T2), with standard therapy plus 200 mg/day apple extract, had mean NF-κB levels decline from 3.500 ± 2.053 pg/mL pre-treatment to approximately 2.666 ± 1.585 pg/mL post-treatment. Treatment group 3 (T3), administered 200 mg/day apple extract alone, showed a decrease from about 3.027 ± 1.437 pg/mL to 2.381 ± 0.934 pg/mL (**Figure 2(A)**). These findings suggest that higher doses of apple extract may exert a greater influence on decreasing systemic NF-κB, a key mediator of inflammation. These findings suggest that higher doses of apple extract may exert a greater influence on decreasing systemic NF-κB, a key mediator of inflammation.

Similarly, IL-6 concentrations decreased 3.25pg/mL in C+, 4.47 pg/mL in T1, 2.95 pg/mL in T2, and 3.98 pg/mL in T3 (**Figure 2(B)**). TNF-α levels declined minimally 6.18 pg/mL in C+, 7.86 pg/mL in T1, 3.69 pg/mL in T2, and 3.82 pg/mL in T3 (**Figure 2(C)**), whereas IL-8 decreases were approximately 14.50 pg/mL, 13.76 pg/mL, 22.22 pg/mL, and 16.75 pg/mL, respectively (**Figure 2(D)**). Although overall changes lacked statistical significance, the data suggest a possible trend toward greater reductions in the T2 and T3 groups, especially for NF-κB and IL-8.

Mechanistically, quercetin-rich apple extract is known to inhibit NF-κB activation, a pivotal transcription factor that regulates the expression of pro-inflammatory cytokines, including IL-6, TNF-α, and IL-8. The observed downward trends align with quercetin's proposed mechanism of action, which attenuates NF-κB-mediated inflammatory signaling and oxidative stress pathways, potentially disrupting the chronic inflammation cycle characteristic of persistent allergic rhinitis. These findings suggest that, although not statistically significant in this limited cohort and treatment duration, apple extract supplementation may be associated with modest changes in key molecular drivers of inflammation.

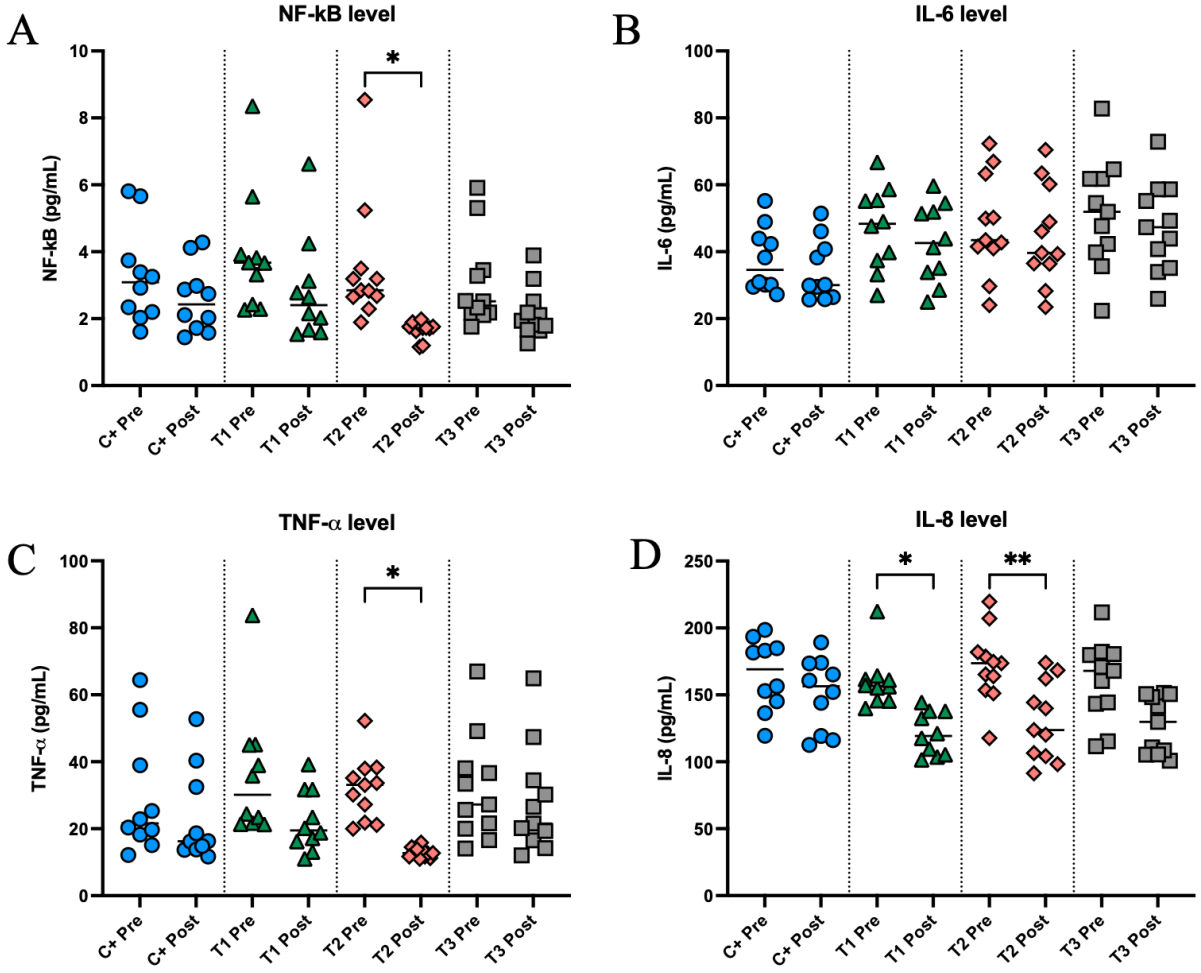


Figure 2 Serum levels of inflammatory biomarkers before and after two weeks of treatment in persistent allergic rhinitis patients. (A) NF-κB, (B) IL-6, (C) TNF-α, and (D) IL-8 levels were measured by enzyme-linked immunosorbent assay (ELISA) in 4 groups. Data are presented as mean ± standard error of the mean (SEM) with individual data points shown. No statistically significant differences were observed between pre-treatment (Pre) and post-treatment (Post) values within any group ($p > 0.05$).

Serum malondialdehyde (MDA) levels, a biomarker of systemic oxidative stress, were measured in the control group (C+), which received standard treatment, and decreased from 7.450 ± 2.105 ng/mL pre-treatment to 5.754 ± 2.240 ng/mL post-treatment. Treatment group 1 (T1), which received standard therapy plus 100 mg/day of apple extract, demonstrated a reduction from around 9.71 ± 3.105 ng/mL to 8.20 ± 1.140 ng/mL. Treatment group 2 (T2), treated with standard therapy plus 200 mg/day apple extract, showed a decrease from roughly 10.365 ± 1.573 ng/mL to 8.372 ± 2.024 ng/mL. Treatment group 3 (T3), receiving 200 mg/day apple extract alone, presented a mean MDA reduction from about 7.399 ± 3.162 ng/mL to 6.321 ± 2.241 ng/mL (**Figure 3**). Despite these downward trends, the differences between pre- and post-treatment

MDA levels were not statistically significant in any group. Nevertheless, the T2 group, which combined the highest apple extract dose with standard therapy, exhibited the greatest absolute reduction in MDA. This pattern is consistent with the proposed antioxidant mechanism of quercetin-rich apple extract, which is thought to mitigate oxidative stress by scavenging reactive oxygen species and inhibiting lipid peroxidation. Collectively, these findings suggest a nonsignificant trend toward reduced oxidative burden with apple extract supplementation.

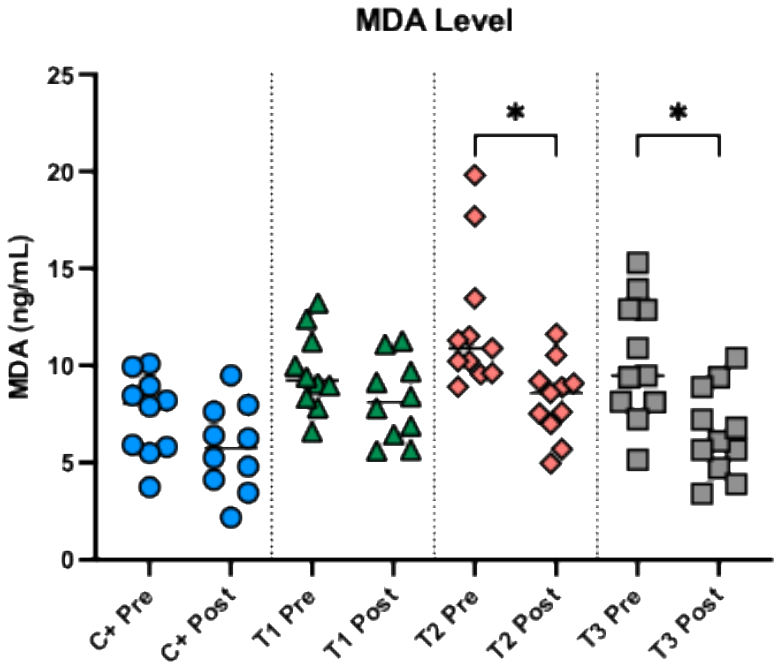


Figure 3 Serum levels of MDA oxidant biomarkers before and after 2 weeks of treatment in persistent allergic rhinitis patients were measured by enzyme-linked immunosorbent assay (ELISA) in four groups. Data are presented as mean ± standard error of the mean (SEM) with individual data points shown. No statistically significant differences were observed between pre-treatment (Pre) and post-treatment (Post) values within any group ($p > 0.05$).

Clinical symptoms

Baseline Total Nasal Symptom Score (TNSS) scores showed no significant differences between groups ($p = 0.562$). Post-treatment analysis revealed significant improvements in all apple extract groups. Most notable improvement occurred in T3 (apple extract alone): Baseline 3.00 ± 0.00 to post-treatment 1.00 ± 0.00 ($p = 0.002$). Inter-group comparison of symptom improvement (Δ) showed significant differences ($p = 0.009$), with T3 demonstrating superior efficacy (Δ = 2.00 ± 0.00) (**Figure 4**).

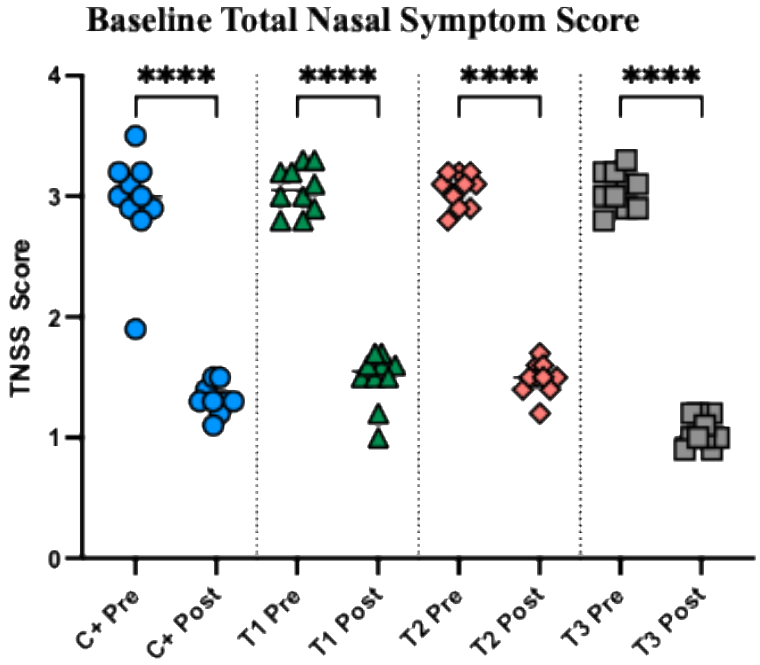


Figure 4 Changes in Total Nasal Symptom Score (TNSS) at baseline and post-treatment across groups. Data are presented as mean ± standard error of the mean (SEM) with individual data points shown. *Statistically significant differences were observed between pre-treatment (Pre) and post-treatment (Post) values within any group (p <0.05).

The clinical improvements observed in the Total Nasal Symptom Score (TNSS), particularly in group T3, suggest that apple extract supplementation may have contributed to symptomatic relief in patients with persistent allergic rhinitis [22,31]. Although biochemical markers such as NF-κB and cytokines did not change significantly, the consistent downward trends across treatment groups may suggest potential biological activity of the quercetin-rich apple extract. The apparent beneficial effects of the apple extract monotherapy (T3) could reflect quercetin's broad bioactive properties, as suggested by prior in vitro and animal studies. Quercetin has been reported to modulate several pathways implicated in allergic inflammation, including mast cell stabilization via calcium channel regulation, modulation of Th1/Th2 immune responses through STAT signaling [32,33], and antioxidant activity that reduces oxidative stress-driven inflammation [34]. While these mechanisms were not directly assessed in this study, the observed biochemical and clinical trends are consistent with these previously described effects. Although TNF-α, IL-6, and IL-8 are nonspecific inflammatory markers, their downward trends may indicate a possible anti-inflammatory effect of apple extract. The nonsignificant reductions in malondialdehyde (MDA) levels across all treatment groups suggest a possible attenuation of lipid peroxidation, consistent with prior evidence that quercetin can reduce oxidative stress in inflammatory conditions [35]. Such antioxidant effects may contribute to improved mucosal health and reduced inflammatory burden in allergic rhinitis.

An important limitation of this study is the short 14-day intervention period, which may be insufficient to assess sustained immunological and biochemical effects in persistent allergic rhinitis. The brief duration limits the evaluation of whether the observed biomarker changes are durable or merely transient. Longer intervention and follow-up periods are therefore needed to determine the long-term efficacy of quercetin-rich apple extract in PAR. The small sample size of 10 participants per group further limits the statistical power to detect changes in biochemical biomarkers, particularly those with high inter-individual variability. Chronic inflammatory conditions like PAR involve sustained immune dysregulation and oxidative stress, which typically require prolonged therapy to achieve meaningful modulation [21,28]. The short timeframe may have been sufficient only to capture early symptomatic relief or transient biomarker fluctuations

rather than stable immunological changes [36]. Longer treatment durations are likely necessary to evaluate whether the observed improvements in TNSS and biomarker trends are durable or clinically significant over time. In this study, the dose-response relationship observed, with optimal effects at 200 mg daily, likely reflects saturable binding kinetics at key molecular targets, including NF-κB regulatory proteins, cytokine receptors, and antioxidant enzyme systems. Higher doses beyond this threshold may not provide proportional benefits due to receptor saturation and metabolic limitations in quercetin bioavailability [20,23]. Furthermore, the optimum efficacy of monotherapy with apple extract compared to combination treatments may indicate that conventional medications can interfere with quercetin's molecular mechanisms by competitive binding at shared targets or by metabolic interactions that limit bioactive quercetin concentrations at tissue sites.

The biomarker data in our study showed nonsignificant downward trends in NF-κB, cytokines, and oxidative stress markers, consistent with quercetin's proposed pleiotropic anti-allergic effects [30,37]. The observed decreases in TNF-α, IL-6, and IL-8 may suggest that quercetin could simultaneously influence multiple inflammatory amplification loops, preventing cross-talk between innate and adaptive immune responses that sustains allergic rhinitis pathology [22]. TNF-α reduction interrupts the early inflammatory cascade that triggers downstream cytokine production, while IL-6 suppression limits Th2 cell differentiation and B cell activation pathways, which are critical for IgE-mediated allergic responses [22,38].

The reduction in IL-8, especially in groups T1 and T2, suggests a possible inhibitory effect on neutrophil recruitment, although this finding was not statistically significant, thereby preventing tissue damage and chronic inflammation that characterize persistent allergic rhinitis [39,40]. These molecular changes collectively suggest that quercetin functions as a master regulator of allergic inflammation, simultaneously targeting multiple pathophysiological mechanisms through its unique polyphenolic structure, which enables diverse protein-binding interactions and cellular penetration.

Additional study limitations include the small sample size, which reduces power for biomarker analyses, and the absence of local tissue biomarker assessments (e.g., nasal secretions), which could have provided stronger evidence of mucosal-level anti-inflammatory activity. Future trials should extend the duration of supplementation, include serial measurements of cytokine and oxidative stress markers, and incorporate mechanistic endpoints, such as STAT pathway activation, mast cell mediators, and analyses of the Th1/Th2 ratio, to clarify the pathways through which quercetin exerts its potential benefits in chronic allergic inflammation.

Conclusions

In conclusion, quercetin-rich apple extract may be associated with clinical improvement in patients with persistent allergic rhinitis, while changes in inflammatory and oxidative stress biomarkers showed only nonsignificant downward trends. These findings suggest a potential therapeutic effect, but further studies with larger sample sizes, longer treatment duration, and quantitative phytochemical standardization are needed to confirm these results. These findings contribute to the growing body of evidence supporting the use of natural anti-inflammatory compounds in the management of allergic diseases and offer patients effective, well-tolerated therapeutic alternatives.

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Declaration of generative AI in scientific writing

The author utilized artificial intelligence tools solely for grammar and language editing purposes to enhance readability, as the author is not a native English speaker. The AI was not used for data analysis, interpretation, or content generation. The author takes full responsibility for the accuracy, integrity, and originality of all content presented in the manuscript.

CRediT author statement

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