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Renoprotective Effects of Olive Leaf Extract on Kidney Function and Histopathology in Ovalbumin-Induced Asthmatic BALB/c Mice

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ABSTRACT

Purpose of the study: This study aimed to evaluate the potential of olive leaf extract–based herbal therapy on renal structure and function in BALB/c mice with experimentally induced asthma.

Methodology: A true experimental laboratory design was conducted using 24 male BALB/c mice divided into four groups: normal control, asthma control, low-dose (100 mg/kgBW), and high-dose (200 mg/kgBW) olive leaf extract treatment. Asthma was induced via ovalbumin sensitization and challenge. Serum creatinine, blood urea nitrogen (BUN), and kidney histopathology were assessed.

Main Findings: Asthma induction significantly elevated creatinine (0.61 ± 0.07 mg/dL) and BUN (29.7 ± 3.4 mg/dL) levels and caused moderate renal histopathological damage (median 2 [2–3]). Treatment with olive leaf extract reduced creatinine to 0.48 ± 0.06 mg/dL and 0.45 ± 0.05 mg/dL, BUN to 22.6 ± 2.8 mg/dL and 20.9 ± 2.5 mg/dL, and histopathological scores to median 1 (1–2) and 1 (0–1) for low- and high-dose groups, respectively.

Novelty/Originality of this study: This study provides the first integrative evaluation of renal protection by olive leaf extract in asthma-induced systemic inflammation, highlighting both functional and structural benefits at therapeutic doses.

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1. INTRODUCTION

Herbal medicine continues to gain global recognition as an integral component of health care systems [1]-[3]. The World Health Organization estimates that approximately 70% of the world's population has used plant-based therapies for the prevention or treatment of disease [4], [5]. In Indonesia, herbal preparations are formally categorized into *jamu*, standardized herbal medicine, and phytopharmaceuticals, reflecting an increasing effort to align traditional remedies with modern scientific validation [6]. Despite widespread acceptance, concerns regarding safety, dosage standardization, and organ-specific toxicity remain inadequately addressed, particularly when herbal products are used in chronic inflammatory diseases such as asthma.

Asthma is a systemic inflammatory disorder characterized not only by airway hyperresponsiveness but also by persistent immune activation that may influence extra-pulmonary organs [7], [8]. While therapeutic strategies primarily target bronchial inflammation, emerging evidence suggests that chronic inflammatory conditions can induce oxidative stress and microvascular alterations in distant organs, including the kidney [9].

However, most preclinical asthma studies focus exclusively on pulmonary outcomes, leaving the potential structural and functional consequences on renal tissue largely unexplored [10]. This gap is clinically relevant, considering that long-term pharmacological management of asthma may involve agents with nephrotoxic potential, and that systemic inflammation itself may predispose to renal dysfunction.

Among promising phytotherapeutic agents, olive leaf extract derived from Olea europaea has attracted increasing scientific interest [11], [12]. Its principal bioactive compounds, oleuropein and hydroxytyrosol, exhibit strong antioxidant, anti-inflammatory, and immunomodulatory properties [13]. Experimental studies have demonstrated that oleuropein can suppress mast cell degranulation and downregulate Th2-mediated cytokines such as IL-4 in bronchial inflammation models [14], [15]. These findings support the potential utility of olive leaf extract as an adjunctive therapy in asthma management [16], [17]. Nevertheless, safety evaluation remains a critical issue. High concentrations of olive leaf extract have been reported to induce hepatotoxic and nephrotoxic effects in animal models, indicating a dose-dependent dualistic profile protective at therapeutic levels yet potentially harmful when improperly administered.

From a pharmacokinetic perspective, all administered compounds undergo absorption, distribution, metabolism, and excretion processes, with the kidney serving as the primary organ responsible for eliminating xenobiotics and their metabolites [18]. Consequently, renal tissue represents a vulnerable target for both protective and toxic effects of herbal compounds . Despite extensive research on the pulmonary benefits of olive leaf extract, limited evidence exists regarding its impact on renal morphology and function under inflammatory conditions such as experimental asthma. This represents a significant research gap: whether olive leaf extract exerts nephroprotective, neutral, or adverse effects when administered as part of asthma therapy remains unclear.

The present study addresses this critical gap by investigating the potential of olive leaf extract–based herbal therapy on structural and functional changes in the kidneys of BALB/c mice with experimentally induced asthma. By integrating histopathological assessment and renal function biomarkers at graded doses (100 mg/kgBW and 200 mg/kgBW), this research seeks to clarify the safety profile and systemic implications of olive leaf extract cultivated in Indonesia.

The novelty of this study lies in its integrative approach: rather than examining pulmonary outcomes alone, it evaluates the kidney as a non-pulmonary target organ within the context of asthma therapy. Furthermore, it explores the dose-dependent renal response to olive leaf extract under inflammatory stress conditions, an area scarcely addressed in prior literature. The urgency of this investigation stems from the escalating use of herbal medicines in chronic diseases without comprehensive organ-specific safety validation. Establishing evidence regarding renal structural integrity and functional status following olive leaf extract administration is essential to support its rational, safe, and scientifically grounded application in asthma management.

2. RESEARCH METHOD

2.1 Study Design and Experimental Setting

This study employed a true experimental laboratory design using a post-test control group approach to evaluate the effect of olive leaf extract–based herbal therapy on renal structure and function in BALB/c mice with experimentally induced asthma. The research was conducted in an accredited biomedical laboratory under controlled environmental conditions (temperature 22–25°C, humidity 50–60%, 12-hour light–dark cycle). All experimental procedures adhered to institutional ethical standards for animal research and were approved by the Institutional Animal Care and Use Committee (IACUC).

Male BALB/c mice (8–10 weeks old; body weight 20–30 g) were selected due to their well-documented Th2 immune response, making them suitable for allergic asthma modeling. Animals were acclimatized for seven days prior to intervention and provided standard pellet diet and water ad libitum [19], [20]. The minimum sample size was determined using Federer's formula for experimental animal studies, ensuring adequate statistical power. Mice were randomly allocated into four groups (n = 6 per group). Randomization was performed using a computer-generated random number table to minimize selection bias. Before presenting the treatment distribution, the grouping structure is summarized in Table 1.

Table 1. Experimental Group Allocation and Intervention Protocol

Group	Asthma Induction	Olive Leaf Extract Dose	Description
K1 (Normal Control)	No	None	Healthy mice without asthma induction or extract
K2 (Asthma Control)	Yes	None	Asthma-induced mice without extract therapy
P1 (Low Dose)	Yes	100 mg/kgBW	Asthma-induced mice treated with olive leaf extract

P2 (High Dose)	Yes	200 mg/kgBW	Asthma-induced mice treated with olive leaf extract

Table 1 illustrates the experimental framework designed to compare baseline renal parameters, asthma-induced alterations, and dose-dependent responses to olive leaf extract therapy.

2.2 Induction of Experimental Asthma

Asthma was induced using an ovalbumin (OVA)-sensitization and challenge protocol, a widely accepted method for generating allergic airway inflammation in BALB/c mice [21]. Sensitization was performed via intraperitoneal injection of OVA (20 µg) emulsified in aluminum hydroxide on days 0 and 7. Aerosolized OVA (1%) exposure was conducted for 20 minutes daily from days 14 to 21 using a nebulization chamber. Successful induction was confirmed clinically through observation of respiratory distress and immunologically via elevated eosinophilic response [22].

Olive leaves were obtained from locally cultivated Olea europaea grown in Indonesia. Botanical identification and authentication were conducted by a certified plant taxonomist. The leaves were air-dried, powdered, and subjected to ethanol extraction using a maceration method. The extract was filtered, concentrated using a rotary evaporator, and stored at 4°C until use. Phytochemical screening confirmed the presence of phenolic compounds, including oleuropein and hydroxytyrosol. Extract doses of 100 mg/kgBW and 200 mg/kgBW were selected based on prior safety and efficacy studies. Administration was performed orally via gastric gavage once daily from day 14 to day 28.

At the end of the intervention period (day 29), mice were anesthetized, and blood samples were collected via cardiac puncture. Serum was separated through centrifugation at 3000 rpm for 15 minutes. Renal function was evaluated using serum creatinine and blood urea nitrogen (BUN) levels measured with an automated biochemical analyzer.

2.3 Histopathological Examination of Kidney Tissue

Following blood collection, mice were euthanized humanely, and kidneys were harvested immediately. Renal tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4–5 µm thickness, and stained with hematoxylin–eosin (H&E).

Histopathological evaluation focused on:

- Glomerular morphology
- Tubular epithelial integrity
- Interstitial inflammation
- Evidence of necrosis or degeneration

Semi-quantitative scoring was performed by a blinded pathologist to reduce observer bias. The scoring criteria are presented in Table 2.

Table 2. Semi-Quantitative Histopathological Scoring of Renal Damage

Score	Histopathological Criteria
0	Normal renal structure
1	Mild tubular degeneration or minimal inflammatory infiltration
2	Moderate tubular degeneration and interstitial inflammation
3	Severe structural damage, necrosis, or widespread inflammation

Table 2 provides standardized criteria to ensure reproducibility and objectivity in assessing renal structural alterations across groups.

2.4 Data Analysis

All quantitative data obtained from serum creatinine and blood urea nitrogen (BUN) measurements were first entered into a statistical software package and subjected to descriptive analysis [23], [24], [25]. Results were presented as mean ± standard deviation (SD) to describe central tendency and dispersion within each experimental group. Prior to hypothesis testing, the distribution of data was evaluated using the Shapiro–Wilk normality test, considering the relatively small sample size per group. Homogeneity of variance was assessed using Levene's test to determine whether parametric assumptions were fulfilled. These preliminary analyses ensured that the choice of statistical test was methodologically appropriate and reduced the risk of type I or type II errors.

For normally distributed and homogeneous data, intergroup comparisons were analyzed using one-way analysis of variance (ANOVA) to detect overall differences among the four groups. When a statistically significant F value was obtained ($p < 0.05$), post hoc analysis using Tukey's Honestly Significant Difference (HSD) test was performed to identify specific pairwise differences between groups (e.g., asthma control vs.

treatment groups, and low-dose vs. high-dose extract). For data that did not meet parametric assumptions, the non-parametric Kruskal–Wallis test was applied, followed by Dunn's multiple comparison test to determine significant differences between groups. A two-tailed p-value < 0.05 was considered statistically significant for all inferential analyses.

For histopathological scoring data, which were ordinal in nature, results were summarized as median and interquartile range (IQR). Group comparisons were performed using the Kruskal–Wallis test, followed by appropriate post hoc pairwise comparisons when significance was observed. Additionally, correlation analysis (Spearman's rank correlation) was conducted to explore potential associations between renal histopathological scores and serum biomarkers (creatinine and BUN), aiming to determine whether structural alterations were aligned with functional impairment. This comprehensive analytical approach allowed for a rigorous evaluation of dose-dependent effects and strengthened the interpretative validity of the study findings.).

All procedures were conducted in accordance with ARRIVE guidelines to enhance transparency and reproducibility. Randomization, blinded histopathological assessment, standardized dosing, and controlled environmental conditions were implemented to minimize bias. Dose selection was justified based on existing literature, and extract preparation methods were clearly documented to facilitate replication in future studies.
Through this comprehensive methodological approach, the study aims to generate robust and reproducible evidence regarding the renal safety and therapeutic implications of olive leaf extract in the context of experimental asthma.

3. RESULTS AND DISCUSSION

This true experimental laboratory study evaluated the effect of olive leaf extract–based herbal therapy on renal structure and function in BALB/c mice with experimentally induced asthma. Outcomes were analyzed in terms of renal function biomarkers (serum creatinine and BUN) and histopathological changes in kidney tissue across four experimental groups. Serum creatinine levels were measured to assess renal filtration function following asthma induction and olive leaf extract administration. The descriptive and inferential statistical results are presented in Table 3.

Table 3. Serum Creatinine Levels Across Experimental Groups

Group	Mean ± SD (mg/dL)	p-value (ANOVA)
K1 (Normal Control)	0.42 ± 0.05	
K2 (Asthma Control)	0.61 ± 0.07	
P1 (100 mg/kgBW)	0.48 ± 0.06	
P2 (200 mg/kgBW)	0.45 ± 0.05	0.001

Table 3 demonstrates a significant difference in serum creatinine levels among groups (p = 0.001). The asthma control group (K2) showed a marked increase in creatinine compared with the normal control group (K1), indicating impaired renal function following asthma induction. Both treatment groups (P1 and P2) exhibited significantly lower creatinine levels compared with K2 (Tukey post hoc, p < 0.05), suggesting a protective effect of olive leaf extract. The high-dose group (P2) showed creatinine levels approaching normal values, although no statistically significant difference was observed between P1 and P2.

To further evaluate renal functional status, BUN levels were analyzed across groups. The findings are summarized in Table 4.

Table 4. Blood Urea Nitrogen (BUN) Levels Across Experimental Groups

Group	Mean ± SD (mg/dL)	p-value (ANOVA)
K1 (Normal Control)	18.4 ± 2.1	
K2 (Asthma Control)	29.7 ± 3.4	
P1 (100 mg/kgBW)	22.6 ± 2.8	
P2 (200 mg/kgBW)	20.9 ± 2.5	0.000

As shown in Table 4, there was a highly significant difference in BUN levels among groups (p < 0.001). The asthma control group exhibited a substantial increase in BUN compared with the normal control, reflecting renal functional impairment associated with systemic inflammatory stress. Administration of olive leaf extract significantly reduced BUN levels in both treatment groups compared with K2 (p < 0.05). The 200 mg/kgBW dose demonstrated a slightly greater reduction, indicating a dose-responsive trend toward functional improvement.

Histopathological examination was conducted to assess structural alterations in renal tissue. Because histological scoring data were ordinal, results are presented as median (interquartile range) and analyzed using the Kruskal–Wallis test. The findings are shown in Table 5.

Table 5. Semi-Quantitative Histopathological Scores of Renal Damage

Group	Median (IQR)	p-value (Kruskal–Wallis)
K1 (Normal Control)	0 (0–0)	
K2 (Asthma Control)	2 (2–3)	
P1 (100 mg/kgBW)	1 (1–2)	
P2 (200 mg/kgBW)	1 (0–1)	0.000

Table 5 reveals significant differences in renal histopathological scores among groups ($p < 0.001$). The asthma control group demonstrated moderate to severe tubular degeneration, interstitial inflammatory infiltration, and partial glomerular congestion. In contrast, the P1 group showed only mild tubular epithelial degeneration with limited inflammatory cell infiltration. The P2 group exhibited the most preserved renal architecture, with minimal structural alterations comparable to the normal control group. Post hoc analysis confirmed significant differences between K2 and both treatment groups ($p < 0.05$).

To determine whether structural changes corresponded with functional impairment, Spearman's correlation analysis was performed between histopathological scores and serum biomarkers. A strong positive correlation was observed between histopathological scores and serum creatinine ($r = 0.71$, $p < 0.001$), as well as between histopathological scores and BUN levels ($r = 0.76$, $p < 0.001$). These findings indicate that worsening structural damage was directly associated with declining renal function.

Overall, asthma induction significantly impaired renal structure and function, as evidenced by elevated serum creatinine and BUN levels and higher histopathological damage scores. Olive leaf extract administration at both 100 mg/kgBW and 200 mg/kgBW significantly attenuated these alterations. The higher dose demonstrated a more pronounced protective effect, though without evidence of nephrotoxicity at the administered concentration. These results suggest that olive leaf extract exerts a renoprotective effect in BALB/c mice under experimental asthma conditions within the tested dosage range.

This true experimental laboratory study demonstrates that experimental asthma significantly induces renal structural and functional alterations in BALB/c mice, as reflected by elevated serum creatinine and BUN levels accompanied by moderate histopathological damage [26]. Importantly, administration of olive leaf extract at doses of 100 mg/kgBW and 200 mg/kgBW attenuated these alterations in a dose-responsive manner, with the higher dose showing greater preservation of renal architecture and function [27]. These findings indicate that beyond its established anti-inflammatory role in airway pathology, olive leaf extract may exert systemic protective effects on extra-pulmonary organs exposed to chronic inflammatory stress.

The observed increase in creatinine and BUN levels in the asthma control group supports the concept that asthma is not merely a localized airway disorder but a systemic inflammatory condition capable of inducing distant organ involvement. Chronic allergic inflammation is characterized by elevated Th2 cytokines, oxidative stress, and circulating inflammatory mediators, which may compromise renal microcirculation and tubular integrity. Although previous studies have predominantly focused on bronchial inflammation, emerging evidence suggests that prolonged systemic immune activation can disrupt endothelial function and increase oxidative burden in renal tissue. However, prior experimental asthma models have rarely evaluated renal outcomes comprehensively, particularly through combined biomarker and histopathological assessment. This gap in the literature underscores the relevance of the present findings.

The renoprotective effect observed in both treatment groups is biologically plausible and consistent with the known pharmacological properties of bioactive compounds derived from Olea europaea. Oleuropein and hydroxytyrosol possess strong antioxidant and anti-inflammatory activities, including inhibition of reactive oxygen species production and modulation of pro-inflammatory cytokine pathways [28]. Previous investigations have demonstrated that oleuropein suppresses mast cell degranulation and downregulates IL-4 expression in allergic airway models, thereby attenuating bronchial inflammation [29]. However, these studies primarily evaluated pulmonary endpoints and did not extend their analysis to renal tissue under inflammatory stress conditions [30]. The current study therefore expands the scope of olive leaf extract research by demonstrating that its systemic antioxidant capacity may also mitigate secondary renal injury associated with asthma-induced inflammation.

The strong positive correlation between histopathological scores and renal function biomarkers further strengthens the internal validity of the findings. Structural preservation in treatment groups was aligned with improved biochemical parameters, suggesting that olive leaf extract did not merely mask functional impairment but contributed to genuine tissue-level protection. This integrated assessment approach combining biochemical and histological endpoints enhances the robustness of interpretation and reduces the likelihood of isolated parameter bias.

The novelty of this study lies in its integrative and organ-targeted perspective. While olive leaf extract has been widely investigated for its anti-asthmatic and immunomodulatory properties, limited research has evaluated its safety and protective profile on non-pulmonary organs within the context of asthma therapy. Furthermore, concerns regarding potential nephrotoxicity at higher concentrations have been reported in other experimental settings. In contrast, the present findings demonstrate that within the tested therapeutic range (100–200 mg/kgBW), olive leaf extract did not induce nephrotoxic effects but instead conferred protective benefits under inflammatory conditions. This dual evaluation of efficacy and organ safety represents an important contribution to phytopharmacological research, particularly in the context of chronic disease management.

From a clinical and translational standpoint, these findings carry important implications. The growing global use of herbal medicine in chronic inflammatory diseases necessitates comprehensive safety profiling beyond target-organ efficacy. Demonstrating that olive leaf extract may protect renal integrity under systemic inflammatory stress supports its potential development as a complementary therapy in asthma management. Moreover, understanding its systemic impact may guide rational dose selection and reduce the risk of unintended organ toxicity in long-term use.

Despite these strengths, several limitations must be acknowledged. First, the study was conducted in an animal model, and extrapolation to human physiology requires caution. Second, renal oxidative stress markers and inflammatory cytokines were not directly quantified, limiting mechanistic insight into the molecular pathways underlying renoprotection. Third, the intervention period was relatively short, and long-term safety evaluation remains necessary to confirm chronic exposure effects. Finally, only two dosage levels were examined, restricting the ability to define a precise therapeutic window.

4. CONCLUSION

This study demonstrates that experimental asthma significantly impairs renal function and structure in BALB/c mice, as evidenced by increased serum creatinine (0.61 ± 0.07 mg/dL) and BUN levels (29.7 ± 3.4 mg/dL) along with moderate histopathological damage (median score 2 [2–3]) in the asthma control group. Administration of olive leaf extract at doses of 100 mg/kgBW and 200 mg/kgBW effectively attenuated these alterations, reducing serum creatinine to 0.48 ± 0.06 mg/dL and 0.45 ± 0.05 mg/dL, BUN to 22.6 ± 2.8 mg/dL and 20.9 ± 2.5 mg/dL, and histopathological scores to median 1 (1–2) and 1 (0–1), respectively. These findings indicate that olive leaf extract exerts a renoprotective effect in BALB/c mice under experimental asthma conditions, with the higher dose showing more pronounced preservation of renal structure and function. It is recommended to explore long-term safety and dose optimization of olive leaf extract in chronic asthma models and to investigate the underlying molecular mechanisms of its renoprotective effects. The findings provide a basis for potential translational application of olive leaf extract as a complementary therapy in asthma management while safeguarding renal health.).

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