The PHILIPPINE JOURNAL of

Veterinary Medicine

Volume 61 **No. 2** **July – December 2025**

Published by the College of Veterinary Medicine
University of the Philippines Los Baños

The Philippine Journal of Veterinary Medicine

Volume 61 No. 2 July - December 2025

The Philippine Journal of Veterinary Medicine (ISSN 0031-7705 print; eISSN 2984-763X online) is a peer- reviewed international journal of basic, applied, and translational research in veterinary medicine and biomedical science. It is published semi-annually, for the periods January-June and July-December each year, by the College of Veterinary Medicine, University of the Philippines Los Baños. All articles are subjected to double-blind review. Authors of articles appearing in the journal are solely responsible for opinions expressed therein.

Starting in 2023, the Philippine Journal of Veterinary Medicine articles will be available online, and will be browseable and searchable.

All communications should be addressed to:

The Editor-in-Chief
Philippine Journal of Veterinary Medicine
College of Veterinary Medicine
University of the Philippines Los Baños
Laguna, Philippines 4031
Telefax No. +63-49-536-2727
Email: pjvm.uplb@up.edu.ph

This journal is abstracted/indexed by: SCOPUS, Biological Abstracts, Focus on: Veterinary Science & Medicine, Web of Science Zoological Records, CAB Abstracts, Index Veterinarius, Veterinary Bulletin, Parasitology Database, Helminthological Abstracts, Protozoological Abstracts. Review of Medical and Veterinary Entomology, EBSCO, ASEAN Citation Index, Prescopus Russia, i-journal (www.ijournals.my), i-focus (www.ifocus.my), i-future (www.ifocus.my), Philippine E-Journals (https://ejournals.ph) and UPLB Journals Online (http://journals.uplb.edu.ph/index.php/PJVM).

Scan Me
PJVM Latest Articles

Scan Me
PJVM Guidelines for Authors

Scan Me
PJVM Form for Authors

Scan Me
PJVM Form for Reviews

Table of Contents

(Original Research)

Ethanolic Blueberry Extract Inhibits Tubular Injury, Inflammation, and Oxidative Stress in a Mouse Model of Kidney Fibrosis

Ahmad Fauzi[1,a], Nurina Titisari[2,b], Hanirastania[1], Vania Kurniaputri[1], and Dini Agusti Paramanandi[3,c]*

[1]Department of Veterinary Clinical Pathology, Faculty of Veterinary Medicine, Universitas Brawijaya, 65151, East Java, Indonesia
[2]Department of Veterinary Physiology, Faculty of Veterinary Medicine, Universitas Brawijaya, 65151, East Java, Indonesia
[3]Department of Veterinary Histology, Faculty of Veterinary Medicine, Universitas Brawijaya, Malang, East Java 65151, Indonesia

*Corresponding Author: drhfauzi@ub.ac.id (Ahmad Fauzi)

ORCID Numbers: [a]0000-0003-4072-7936 [b]0000-0003-2567-3140 [c]0009-0007-3014-9956

Submitted: 20 May 2025 Revised: 28 Jul. 2025 Accepted: 15 Sept. 2025 Published: 20 Oct. 2025

Abstract

Background: Kidney fibrosis is a progressive condition characterized by tubular injury, inflammatory cell infiltration, and oxidative stress resulting from increased reactive oxygen species. The blueberry exhibits strong antioxidant and anti-inflammatory properties, rendering it a promising natural therapy for inhibiting kidney damage. This study aimed to evaluate the therapeutic effects of this extract on tubular injury scores, the number of inflammatory cells, interleukin-1 beta (IL-1ß) expression, and malondialdehyde (MDA) levels in a Swiss Webster mouse model of kidney fibrosis. **Methods:** An experimental design was conducted using 25 male Swiss Webster mice, divided into five groups: a control group and four groups with unilateral ureteral obstruction (UUO), with or without blueberry extract therapy. The crude 70% ethanolic blueberry (*Vaccinium corymbosum*) extract was administered orally (1500 mg/kg body weight) via gavage for 7 or 14 days**,** followed by histological and biochemical analysis of the harvested kidneys. **Results:** UUO significantly increased tubular injury, inflammatory cell infiltration, IL-1ß expression, and MDA levels compared to the control group. Mice treated with blueberry extract showed a 22–13% reduction in tubular injury scores, a 25–21% decrease in inflammatory cell counts, a 39–34% reduction in IL-1ß expression, and a 7–5% decline in MDA levels at 7 and 14 days, respectively. These therapeutic effects were attributed to the extract's ability to suppress inflammation and inhibit lipid peroxidation triggered by oxidative stress. **Conclusions:** The crude ethanolic extract of *Vaccinium corymbosum* demonstrates significant potential as a natural therapeutic agent in reducing kidney damage, inflammation, and oxidative stress in kidney fibrosis.

Keywords

Vaccinium corymbosum, Kidney fibrosis, Tubular injury, Oxidative stress, IL-1ß marker

1. Introduction

Kidney fibrosis is a progressive pathological condition that contributes significantly to chronic kidney disease and eventually leads to end-stage renal disease. It is characterized by the excessive accumulation of extracellular matrix (ECM) proteins in the renal interstitium, which results in structural damage, impaired kidney function, and irreversible loss of renal tissue [1]. Since fibrosis is a common final pathway in nearly all forms of chronic kidney disease (CKD), it poses a major global health concern due to its rising prevalence and the lack of effective long-term therapies [2]. To better understand and develop

treatments for this condition, researchers often use the unilateral ureteral obstruction (UUO) model in animal studies. This model involves surgically blocking one ureter to mimic the obstructive damage that leads to kidney fibrosis [3]. The resulting pathological changes, such as tubular epithelial injury, immune cell infiltration, and ECM deposition [4], closely resemble those seen in human renal fibrosis, making UUO a reliable and relevant method for evaluating potential therapeutic interventions.

A key process driving the progression of kidney fibrosis is chronic inflammation. Injury to renal tissue activates pro-inflammatory signaling pathways and stimulates the release of cytokines like interleukin-1β (IL-1β), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) [5]. These cytokines are produced by resident renal cells and infiltrating immune cells, including macrophages and lymphocytes, in response to injury. The accumulation of these inflammatory mediators promotes the recruitment of additional immune cells, leading to a sustained inflammatory response [6]. Inflammation is linked to oxidative stress, where reactive oxygen species (ROS) are produced in excess, leading to lipid peroxidation, protein degradation, and DNA damage [7]. In the UUO model, ischemia and cellular injury cause elevated ROS generation [8], which enhances inflammatory pathways, stimulates fibroblast activation, and ECM deposition [9]. One of the by-products of lipid peroxidation is malondialdehyde (MDA), a reactive aldehyde commonly used as a biomarker for oxidative stress [10]. Elevated MDA levels in renal tissue indicate ongoing peroxidative damage to cell membranes and are associated with disease severity in kidney fibrosis [11]. Evaluating MDA concentration provides insight into the oxidative stress in the renal environment and the effectiveness of antioxidant therapies.

Given the key roles of inflammation and oxidative stress in kidney fibrosis, therapeutic strategies that target these mechanisms hold considerable promise [12]. Synthetic drugs have shown some effectiveness, but their long-term use is limited by adverse effects. Natural products with antioxidant and anti-inflammatory properties, such as blueberries (*Vaccinium spp.*), have gained interest due to their rich anthocyanin content, strong bioactive flavonoids [13,14]. This study investigates whether a crude ethanolic extract of blueberry can protect against UUO-induced kidney damage in mice by reducing tubular injury, suppressing IL-1β expression, lowering MDA levels, and ultimately attenuating fibrosis. The findings may support the use of natural dietary compounds in managing chronic renal diseases and improving therapeutic outcomes.

2. Materials and Methods

2.1 Blueberry extract preparation

Blueberry extract used in this study was prepared by maceration, a method known for effectively isolating bioactive compounds from plant material. A total of 500 grams of air-dried blueberries (*V. corymbosum)*, purchased from PT. Mustika Karya Anugrah, a certified supplier in Tangerang, Indonesia) were immersed in 2500 mL of 70% ethanol (Merck®, analytical grade, 96% ethanol diluted with distilled water). The maceration process lasted 72 hours at room temperature, allowing anthocyanins and other phenolic compounds to dissolve into the solvent. Following this, the solution underwent evaporation using a rotary evaporator (Buchi R-300, Switzerland) at 40°C to remove the ethanol and concentrate the extract. The final extract was re-dissolved in 0.5% carboxymethylcellulose (CMC) as a vehicle before oral administration. This procedure followed the optimized extraction method described by Ćujić *et al.* (2016), ensuring maximum retention of antioxidant constituents [15].

2.2 Experimental animals and housing conditions

The experimental subjects consisted of 25 male Swiss Webster mice, aged between six to eight weeks, with body weights ranging from 20 to 30 grams. Male mice were selected to minimize hormonal fluctuations present in females, which can influence physiological responses, thereby ensuring uniformity of data. The mice were obtained from a certified laboratory animal supplier and acclimatized for seven days before the experiment began. During the acclimatization period, animals were housed in standard laboratory cages under controlled environmental conditions: a temperature of 22–25°C, relative humidity of 50–60%, and a 12-hour light-dark cycle. Mice were provided with commercial rodent chow (702P, Gold Coin Feedmills Sdn Bhd, Malaysia) and water *ad libitum*. All animal procedures conformed to institutional ethical standards for laboratory animal research.

2.3 Experimental design and procedures

The mice were randomly divided into five groups, each consisting of five animals: Group A: Sham-operated control group (no UUO, no treatment); Group B: UUO-induced kidney fibrosis for seven days (UUO, no treatment); Group C: UUO-induced fibrosis with blueberry extract treatment for seven days (UUO, blueberry extract for seven days); Group D: UUO-induced kidney fibrosis for 14 days (UUO, no treatment); Group E: UUO-induced fibrosis with blueberry extract treatment for 14 days (UUO, blueberry extract for 14 days). This grouping allowed for comparisons between untreated and treated fibrosis, both at early and later stages of disease development.

2.4 Induction of kidney fibrosis: unilateral ureteral obstruction (UUO)

Kidney fibrosis was induced using the unilateral ureteral obstruction (UUO) technique, a widely validated model that mimics progressive renal interstitial fibrosis. Mice were anesthetized with a combination of ketamine (70 mg/kg body weight, bw) and xylazine (15 mg/kg bw), administered intraperitoneally. After confirming adequate anesthesia, the mice were positioned in lateral recumbency, and the right flank area was shaved and disinfected with povidone-iodine.

A 1.5 cm incision was made to expose the right kidney and ureter. The ureter was carefully dissected and ligated at both the proximal and distal ends using 3/0 silk sutures to prevent urine flow. The kidney was then returned to the peritoneal cavity, and the incision was sutured in layers using 2/0 silk thread. Post-operative care included oral administration of ibuprofen as analgesics (30 mg/kg bw) once daily for three days and daily monitoring for signs of distress. This method followed the procedure outlined by Hesketh *et al.* in 2014 [16].

2.5 Blueberry extract administration

Blueberry extract treatment was initiated 24 hours after the UUO procedure. Mice in groups C and E received blueberry extract orally via gavage once daily at a dose of 1500 mg/kg bw, as adapted from the study by Fauzi *et al*. (2020) [17]. Group C received the blueberry extract for seven consecutive days, while group E was treated for 14 days. The dose and duration were chosen based on prior evidence indicating the biological activity of blueberry extract and the development of kidney fibrosis. Groups A, B, and D, which served as the control group, received 10 mL/kg of 0.5% CMC by oral gavage.

2.6 Kidney Harvesting and Tissue Processing

At the end of the respective treatment durations (7 or 14 days), the mice were euthanized by cervical dislocation under anaesthesia. The abdominal cavity was dissected to expose the kidneys. The right kidney from each mouse, which had been subjected to UUO, was harvested and processed for histological and oxidative stress analyses.

For histological evaluation, kidney tissues were rinsed in phosphate-buffered saline (PBS) and fixed in 10% buffered formalin. Fixed tissues were embedded in paraffin and sectioned for staining. A portion of the kidney tissue was also snap-frozen in aluminium foil, stored in labelled tubes, and preserved on ice for MDA level assessment.

2.7 Assessment of tubular injury

Hematoxylin and eosin-stained histological scoring of tubular injury was performed by two independent observers who were blinded to treatment groups to ensure unbiased evaluation. Ten randomly selected non-overlapping fields from each slide were examined under 200x magnification, focusing on the renal cortex. The tubular injury parameters included dilatation, epithelial desquamation, and brush border loss. A semi-quantitative scoring system (Table 1) by Kim *et al.* (2009) was used to grade the severity of injury on a scale from 0 (normal) to 4 (extensive damage, >75% of tubules affected) [18].

2.8 Inflammatory cell quantification

To quantify inflammatory cell infiltration, kidney tissue sections were stained with hematoxylin and eosin and observed under 400x magnification. Mononuclear inflammatory cells were counted in five fields per sample using the

Table 1. Tubular injury scoring scale.

Tubular Injury Score	Description
0	Normal
1.0	Minor, tubular injury < 25%
2.0	Moderate, tubular injury 25–50%
3.0	Severe, tubular injury 50–75%
4.0	Extensive, tubular injury > 75%

Fiji ImageJ software, as described by Permata and Febrianto in 2019 [19]. This provided a reliable estimation of leukocyte infiltration in the renal interstitial area.

2.9 Immunohistochemical Analysis of IL-1β Expression

Interleukin-1 expression in kidney tissues was analyzed using immunohistochemistry. Sections were incubated with IL-1ß Polyclonal Antibody (bs-6319R Rabbit polyclonal, validated for mouse tissue, 1:250 dilution; Bioss®, USA) as the primary antibody. The staining protocol was conducted using the N-Histofine® Simple Stain™ MAX PO (MULTI) kit (Nichirei Biosciences Inc., Japan), which contains polymer-conjugated secondary antibodies and peroxidase. Diaminobenzidine (DAB, 1:40) served as the chromogen substrate, and Mayer's Hematoxylin (1:3) was used for counterstaining. Slides were mounted with Entellan mounting and examined under 400x magnification. Digital images were analyzed using Immunoratio software, which quantified IL-1ß expression as the percentage of DAB-stained nuclear area relative to total nuclear area.

2.10 Measurement of malondialdehyde (MDA) levels

Lipid peroxidation was assessed by determining MDA concentration using the thiobarbituric acid reactive substances (TBARS) method. Approximately 0.15 grams of kidney tissue were homogenized in a 0.9% NaCl solution. The homogenate was mixed with distilled water and 1% sodium thiobarbiturate, followed by centrifugation at 1000 rpm for 10 minutes. The supernatant was collected, and absorbance was measured at 532 nm using a spectrophotometer. MDA concentration was determined based on a standard curve and expressed in ng/mL, following the methodology outlined by Fauziah in 2018 [20].

2.11 Statistical analysis

Quantitative data, including MDA levels, IL-1ß expression, and inflammatory cell counts, were analyzed using One-way Analysis of Variance (ANOVA), followed by Tukey's post hoc test to determine statistical differences between groups. Prior to conducting the ANOVA, the data were tested for normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test), which confirmed that the assumptions were met. Tubular injury scores, being ordinal in nature, were analyzed using the non-parametric Kruskal-Wallis test. Pairwise comparisons were conducted using Dunn's multiple comparison test where appropriate. A significance level of $\alpha = 0.05$ was used for all tests. Data analysis was performed using SPSS software. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1 Macroscopic Kidney Changes

Kidneys of the sham group (Group A), which underwent surgery without ureteral obstruction, appeared normal in size, shape, and color. The kidneys were symmetrical, soft, and displayed a distinctive dark red coloration (Fig. 1), indicative of healthy renal morphology in mice. Meanwhile, untreated kidneys subjected to UUO (Groups B and D) demonstrated clear pathological changes. Specifically, these kidneys appeared enlarged with hydronephrosis, pale, and tense upon gross examination (Fig. 1). Prolonged obstruction for 14 days in Group D resulted in more pronounced discoloration and tissue rigidity. Nevertheless, kidneys treated with blueberry for seven days (Group C) and 14 days (Group E) showed improvement, as evidenced by a decrease in renal volume and the resolution of hydronephrosis features (Fig. 1).

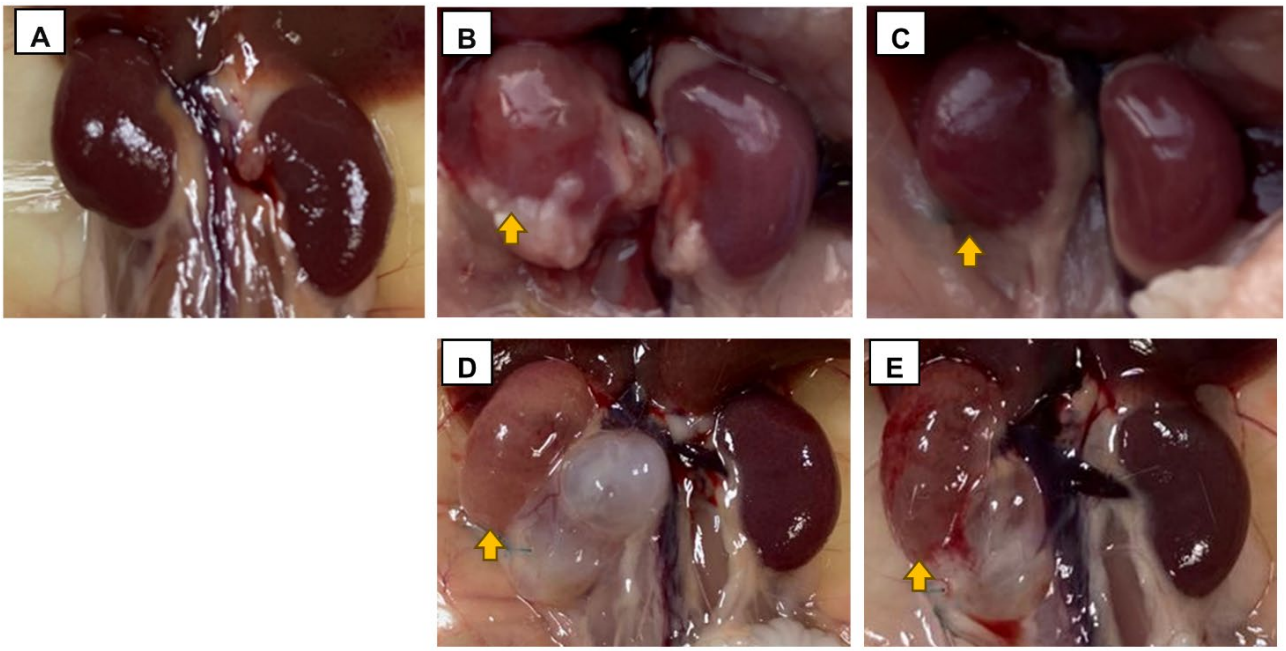

Figure 1. Macroscopic examination of kidneys from UUO-induced mice revealed distinct morphological differences among experimental groups. A) The sham-operated control group exhibited kidneys of normal volume and coloration. B) In contrast, kidneys from UUO-induced mice on day 7 appeared pale and enlarged. C) However, UUO-induced mice treated with blueberry extract for 7 days maintain kidney volume and color similar to the sham-operated control. D) By day 14, UUO-induced mice showed further deterioration, characterized by a pale appearance, approximately double the normal kidney volume, and evident hydronephrosis. E) Interestingly, blueberry treatment for 14 days partially mitigated these effects, resulting in kidneys with reduced hydronephrosis and less pallor than untreated UUO kidneys. Note: Yellow arrows indicate that the right kidneys are subjected to unilateral ureteral obstruction.

3.2 Tubular Injury Scores

The sham group (A) exhibited normal kidney histology, characterized by intact brush borders and no signs of desquamation or dilation (Fig. 2A). Statistical analysis using the Kruskal-Wallis test followed by the Mann-Whitney U test confirmed significant differences among the groups ($p < 0.05$) (Table 2). The UUO-7 group (B) exhibited moderate tubular damage, with an average injury score of 1.78 ± 0.13, which was significantly higher than that of the sham group (A). The severity increased further in the UUO-14 group (D), with a mean score of 3.85 ± 0.13, indicating extensive tubular injury. Groups treated with blueberry extract exhibited reduced injury scores. Group C (UUO + blueberry 7 days) had a mean score of 1.38 ± 0.10, representing a 22.47% decrease compared to Group B. Similarly, Group E (UUO + blueberry 14 days) recorded a score of 3.35 ± 0.06, a 12.99% reduction from Group D.

3.3 Inflammatory Cell Infiltration

The number of inflammatory cells in renal tissue (Table 3) varied significantly (p <0.05) among the experimental groups following UUO induction. Group A (sham-operated control) showed the lowest inflammatory cell count (5.5 ± 2.38), reflecting normal kidney histology. Group B (UUO for seven days) exhibited an increase in inflammatory cells (68.25 ± 11.33), while Group D (UUO for 14 days) had the highest level of infiltration (78.75 ± 6.99). Administration of blueberry extract notably reduced inflammatory cell infiltration. Group C (UUO for seven days + blueberry extract) had an inflammatory cell count of 50.75 ± 7.41, which was significantly lower than Group B. Group E (UUO for 14 days + blueberry extract) had a count of 62.25 ± 6.34, which was substantially lower than Group D.

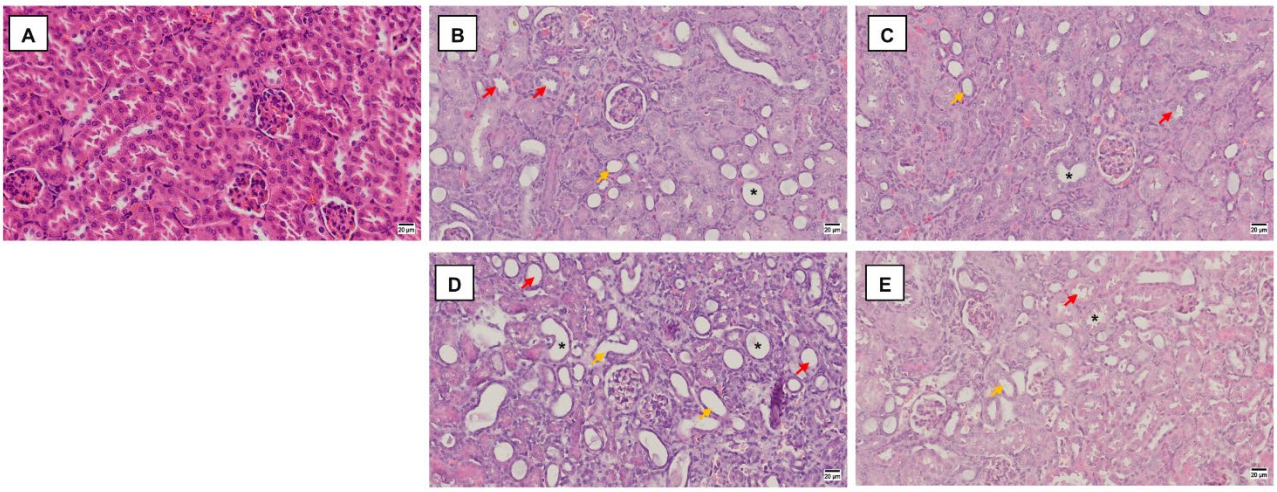

Figure 2. Microscopic evaluation of kidney tissues from UUO-induced mice treated with blueberry extract revealed progressive histological changes. The sham-operated control (A) exhibited normal renal architecture characterized by intact tubules and glomeruli. In contrast, the UUO-induced mice at day 7 (B) showed mildly dilated tubules (*), accompanied by epithelial desquamation (yellow arrow) and noticeable loss of brush borders (red arrow). However, UUO mice treated with blueberry extract for seven days (C) demonstrated improvements, with less dilation of tubules, reduced epithelial desquamation, and preservation of brush borders. By day 14, untreated UUO mice (D) displayed severe renal injury, including tubular necrosis and extensive epithelial loss. Notably, kidneys from mice treated with blueberry extract for 14 days (E) exhibited normal glomerular structures, despite persistent tubular necrosis and epithelial loss. Annotations in the images include dilated tubules (*), epithelial desquamation (yellow arrows), loss of brush borders (red arrows) (H&E staining; 200x magnification; 20 μm scale bar).

Table 2. Tubular injury score in the mouse kidney following the UUO procedure.

Experimental Groups	Tubular Injury Score Mean ± SD
Group A (Sham-operated control group)	0.00 ± 0.00^{a}
Group B (UUO-7 days)	1.78 ± 0.13^{abc}
Group C (UUO-7 days + blueberry extract)	1.38 ± 0.10^{ab}
Group D (UUO-14 days)	3.85 ± 0.13^{c}
Group E (UUO-14 days + blueberry extract)	3.35 ± 0.06^{bc}

Note: Different notations indicate significant differences between treatment groups (p <0.05)

Table 3. Inflammatory cell counts in the mouse kidney following the UUO procedure.

Group	Mean number of inflammatory cells
Group A (Sham-operated control group)	5.5 ± 2.38^{a}
Group B (UUO-7 days)	68.25 ± 11.33^{cd}
Group C (UUO-7 days + blueberry extract)	50.75 ± 7.41^{b}
Group D (UUO-14 days)	78.75 ± 6.99^{d}
Group E (UUO-14 days + blueberry extract)	62.25 ± 6.34^{bc}

Note: Different notations indicate significant differences between treatment groups (p <0.05)

3.4 Interleukin-1β Expression

IL-1β expression in kidney tissue sections increased in UUO-induced mice, and blueberry treatment reduced this expression (Fig. 3). Statistical analysis using the ANOVA, followed by Tukey's post hoc test, confirmed significant differences among the groups (Table 4). The sham-operated control group (Group A) showed the lowest IL-1β expression (9.77 ± 1.85%) among all groups. Group B (UUO for seven days) demonstrated a higher expression (25.03 ± 4.02%) compared to Group A. Further, Group D (UUO for 14 days) showed the highest expression among all groups (36.18 ± 1.52%). Meanwhile, Group C (UUO for seven days + blueberry extract) showed a significantly lower IL-1β expression (15.20 ± 2.34%) compared to Group B. Similarly, Group E (UUO for 14 days + blueberry extract) exhibited markedly reduced IL-1β expression (23.81 ± 1.69%) relative to Group D.

3.5 Malondialdehyde (MDA) Levels

Table 5 presents the MDA levels measured in mouse kidney tissue across all experimental groups. The sham-operated control group (Group A) exhibited the lowest MDA concentration, with a mean value of 364.94 ± 10.20 ng/mL, indicating basal oxidative status under normal conditions. A marked increase in MDA levels was observed in Group B (UUO for seven days), with a mean value of 414.11 ± 3.69 ng/mL, while a further increase was recorded in Group D (UUO for 14 days), which showed the highest MDA level among all groups (448.00 ± 8.56 ng/mL). Administration of blueberry extract was associated with lower MDA levels in both the 7-day and 14-day UUO groups. In Group C (UUO for seven days + blueberry extract), the MDA level was 385.50 ± 1.90 ng/mL, which was markedly reduced compared to Group B. Similarly, Group E (UUO for 14 days + blueberry extract) had an MDA level of 424.94 ± 2.63 ng/mL, which was significantly lower than that of Group D.

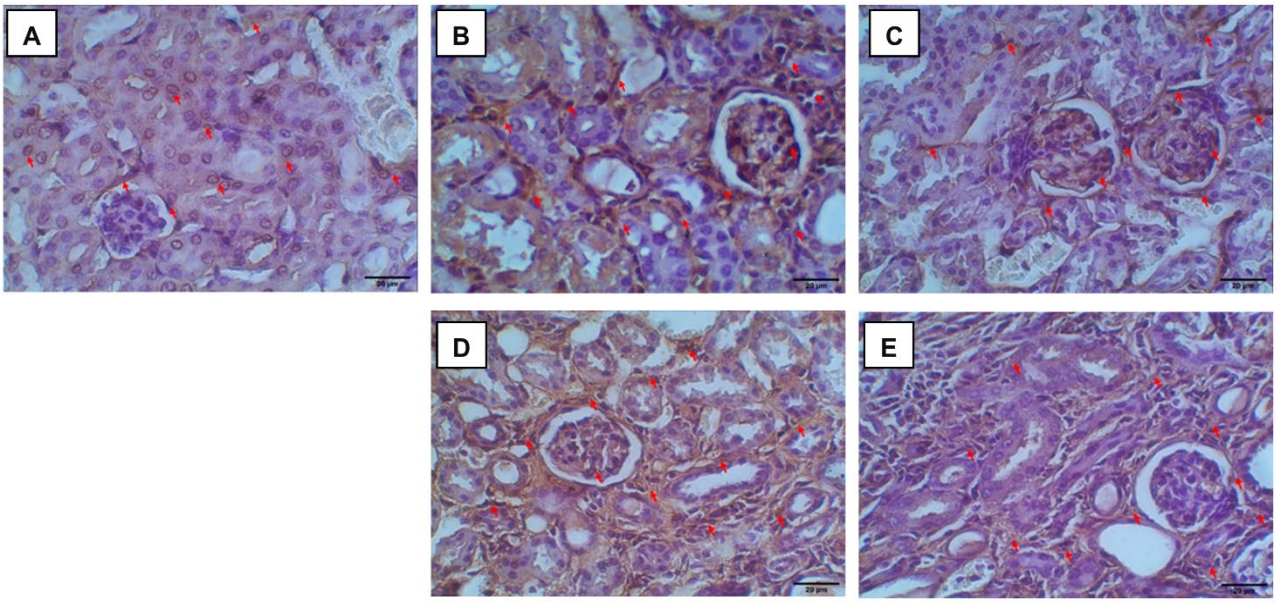

Figure 3. Micrographs illustrating IL-1β expression in the kidneys of UUO-induced mice treated with blueberry extract revealed distinct patterns across experimental groups. The sham-operated control group (A) exhibited minimal IL-1β expression. By day 7, untreated UUO-induced mice (B) demonstrated a mild increase in IL-1β immunoreactivity within glomeruli, renal tubules, and the interstitial areas. Conversely, UUO-induced mice treated with blueberry extract for seven days (C) showed comparatively lower IL-1β expression in these renal compartments. At day 14, untreated UUO-induced mice (D) exhibited severe IL-1β expression, particularly pronounced within interstitial areas, tubules, and glomeruli. However, the group treated with blueberry extract for 14 days (E) displayed substantially reduced IL-1β expression in the interstitial spaces, tubules, and glomeruli. Red arrows in the images specifically highlight IL-1β expression localized in the glomerulus and interstitial tubules. (400x magnification; 20 μm scale bar).

Table 4. Percentage area of IL-1ß expression in the mouse kidney following the UUO procedure.

Group	Mean IL-1β Expression (% area)
Group A (Sham-operated control group)	9.77 ± 1.85^{a}
Group B (UUO-7 days)	25.03 ± 4.02^{c}
Group C (UUO-7 days + blueberry extract)	15.20 ± 2.34^{b}
Group D (UUO-14 days)	36.18 ± 1.52^{d}
Group E (UUO-14 days + blueberry extract)	23.81 ± 1.69^{c}

Note: Different notations indicate significant differences between treatment groups ($p < 0.05$)

Table 5. MDA expression in the mouse kidney following the UUO procedure.

Group	Mean MDA Levels (ng/mL)
Group A (Sham-operated control group)	364.94 ± 10.20^{a}
Group B (UUO-7 days)	414.11 ± 3.69^{c}
Group C (UUO-7 days + blueberry extract)	385.50 ± 1.90^{b}
Group D (UUO-14 days)	448.00 ± 8.56^{d}
Group E (UUO-14 days + blueberry extract)	424.94 ± 2.63^{c}

Note: Different notations indicate significant differences between treatment groups ($p < 0.05$)

4. Discussion

This study investigated the therapeutic potential of an ethanolic extract of blueberry (*V. corymbosum*) in mitigating kidney damage induced by unilateral ureteral obstruction (UUO), a well-established animal model of renal fibrosis. Previous studies have established that UUO-induced animals develop kidney inflammation within three days, followed by histological alterations in one week, and develop into kidney fibrosis within two weeks [8]. In addition, a study reported that UUO induces tubular damage, macrophage infiltration, and interstitial fibrosis through upregulation of pro-inflammatory cytokines and oxidative pathways [21,22]. In this current study, findings demonstrated that blueberry extract significantly reduced tubular injury, inflammatory cell infiltration, IL-1ß expression, and MDA levels, suggesting its effectiveness in attenuating both inflammatory and oxidative pathways involved in the pathogenesis of kidney fibrosis.

Kidney fibrosis is a complex process characterized by excessive accumulation of extracellular matrix, tubular epithelial injury, and persistent inflammation [23]. UUO-induced kidney fibrosis mimics these conditions by mechanically obstructing urinary flow, resulting in ischemia, tubular damage, and inflammatory activation [24]. The observed macroscopic changes in the UUO groups, including enlarged and pale kidneys, confirm successful model induction. These morphological alterations reflect hydronephrosis and progressive fibrotic remodeling, which are consistent with earlier studies reporting progressive hydronephrosis and renal parenchymal loss in UUO models [25,26].

Histological analysis further supported these observations. In untreated UUO groups, particularly after 14 days, severe tubular injury was evident, including epithelial desquamation, tubular dilatation, and loss of the brush border. These are hallmarks of early and progressive fibrotic damage and are consistent with the ischemic injury described by Bonventre and Yang in 2011[27]. In contrast, mice treated with blueberry extract displayed notably reduced injury scores, especially in the 7-day treatment group. This suggests that the bioactive compounds in the crude extract may help preserve tubular integrity by counteracting oxidative and inflammatory insults at an early stage of fibrosis progression.

A major component in the pathogenesis of kidney fibrosis is the infiltration of mononuclear inflammatory cells, particularly macrophages and lymphocytes. These cells perpetuate tissue damage by releasing pro-inflammatory and pro-fibrotic cytokines. The significant increase in inflammatory cells observed in UUO kidneys aligns with previous reports that chronic inflammation plays a pivotal role in fibrosis [3,28]. Notably, administration of blueberry extract resulted in a substantial reduction in inflammatory cell counts, indicating that the extract effectively inhibited immune cell recruitment. This anti-inflammatory effect is consistent with prior reports on anthocyanin-rich blueberry extracts reducing inflammation and leukocyte infiltration [29].

The role of IL-1β as a pro-inflammatory cytokine was further emphasized in this study. IL-1β expression was significantly elevated in UUO groups and closely correlated with the extent of injury and inflammation. As a central mediator of inflammation, IL-1β is known to induce leukocyte recruitment, fibroblast activation, and extracellular matrix deposition [31,32]. The observed suppression of IL-1β expression in the blueberry-treated groups reinforces the conclusion that anthocyanins not only reduce leukocyte infiltration but also modulate the inflammatory cascade at a molecular level, as stated by several studies [33,34]. The mechanisms likely involve inhibition of ROS-induced inflammasome activation and reduced maturation of IL-1β from its precursor forms [35,36]. These findings are of particular relevance given that persistent IL-1β expression has been associated with chronic and progressive fibrosis [36].

Oxidative stress is another key contributor to renal fibrosis. UUO impairs renal perfusion, leading to hypoxia and excessive production of ROS. These molecules initiate lipid peroxidation, mitochondrial dysfunction, and cell apoptosis, thereby accelerating fibrotic changes [37]. The elevated MDA levels in UUO groups in this study reflect this ongoing oxidative damage. MDA, as a stable by-product of lipid peroxidation, serves as a reliable indicator of oxidative stress in renal tissues [38]. Treatment with blueberry extract significantly reduced MDA concentrations, indicating that the extract limited ROS production and protected lipid membranes from oxidative degradation. This antioxidant effect is consistent with the chemical properties of anthocyanins, which act as free radical scavengers and metal chelators [40,41].

The active compounds in blueberries, particularly delphinidin and malvidin, are known for their high radical-scavenging capacity [42,43]. In addition to directly neutralizing ROS, anthocyanins may also enhance the activity of endogenous antioxidant enzymes such as superoxide dismutase and catalase, further contributing to cellular defense [43]. The reduced MDA levels in the treatment groups, particularly in the 7-day after UUO surgery, highlight the potential of blueberry extract to attenuate oxidative damage in early-stage fibrosis. However, in the 14-day treatment group, while improvements were still significant, the effect size was smaller, possibly due to more extensive and irreversible damage at later stages of fibrosis. Indeed, during the later stages of fibrosis, this process becomes increasingly dysregulated and self-perpetuating, resulting in extensive and irreversible damage to the renal parenchyma [44].

Interestingly, across all parameters—tubular injury, inflammation, IL-1β expression, and MDA levels—both 7-day and 14-day treatments are effective, with slightly higher improvement observed on day seven. This finding suggests that earlier intervention yields greater benefits, likely because fibrosis is more responsive to antioxidant and anti-inflammatory therapies during the initial phase, before irreversible ECM deposition and tissue remodeling have occurred. Once fibrosis becomes entrenched, therapeutic agents may have limited efficacy in reversing structural changes, even if they continue to modulate inflammation or oxidative stress. A study indicated that the early phase of fibrosis is more responsive to therapy due to tissue repair dynamics, while the advanced phase of fibrosis creates a "mis-instructive" environment that activates fibrogenic cells and strengthens the pathological loop through tissue stiffness and TGF-β1 activation, making therapy difficult and often irreversible [45]. These findings underscore the importance of timely intervention in fibrotic kidney diseases and suggest that crude blueberry extract may be most effective when administered in the early stages of disease progression.

5. Conclusions

In summary, the results of this study support the hypothesis that crude ethanolic blueberry (*V. corymbosum*) extract possesses renoprotective properties in the context of obstructive kidney injury. The bioactive compounds within the extract may act through multiple mechanisms, including suppression of inflammation, modulation of cytokine expression, and inhibition of oxidative damage. These effects collectively contribute to reduced tissue injury and fibrosis. Since the extract used was crude and its phytochemical components were not quantified, further studies are needed to isolate and identify the bioactive compounds. Future research should expand upon these findings by investigating the molecular pathways modulated by its phytochemical compounds in greater detail, exploring dose-response relationships, and evaluating long-term outcomes. In addition, comparative studies involving other natural antioxidants could help contextualize the efficacy of blueberry extract within the broader spectrum of phytotherapeutic interventions. Given the increasing global burden of chronic kidney disease and the limitations of current pharmacological treatments, such natural compounds offer promising, low-toxicity alternatives or adjuncts to existing therapies.

Availability of Data and Materials

All data are available in this study

Author Contributions

Conceptualization, A.F., and N.T.; Methodology, A.F., N.T., and D.A.P.; Investigation, A.F., H.S., and V.K.; Data curation, H.S., and V.K.; Writing – Original Draft, A.F.; Writing – Review and Editing, N.T., and D.A.P.; Funding Acquisition, A.F.; Supervision, N.T., and D.A.P.

Ethics Approval and Consent to Participate

The Institutional Animal Care and Use Committee (IACUC) at Universitas Brawijaya, under number 045.KEP-UB.

Acknowledgment

The authors would like to express their sincere gratitude to the Faculty of Veterinary Medicine, Universitas Brawijaya, for the continuous support provided during this study. We also wish to extend our appreciation to the staff of the Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Universitas Brawijaya, and the staff of the Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya, for their valuable technical assistance and contributions that greatly facilitated the completion of this research.

Funding

This work was financially supported by the Directorate of Research and Community Service (DRCS), Universitas Brawijaya, under Grant No.: 436.31/HPP.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Huang, R., Fu, P., & Ma, L. (2023) Kidney fibrosis: From mechanisms to therapeutic medicines. *Signal Transduct Target Therapy, 8(1)*,129. https://doi.org/10.1038/s41392-023-01379-7.

[2] Reiss, A.B., Jacob, B., Zubair, A., Srivastava, A., Johnson M., & De Leon, J. (2024) Fibrosis in chronic kidney disease: Pathophysiology and therapeutic targets. *Journal of Clinical Medicine, 13(7).* https://doi.org/10.3390/jcm13071881.

[3] Martínez-Klimova, E., Aparicio-Trejo, O.E., Tapia E., & Pedraza-Chaverri, J. (2019) Unilateral ureteral obstruction as a model to investigate fibrosis-attenuating treatments. *Biomolecules, 9(4).* https://doi.org/10.3390/biom9040141.

[4] Sulistiyowati, I., Yunus J., Sari D.C.R., & Arfian, N. (2020) Upregulation of p16, bax

and bcl-2 mrna expression associated with epithelial apoptosis and myofibroblast proliferation in kidney fibrosis model in mice. *Malaysian Journal of Medical Sciences, 27(2)*,37–44. https://doi.org/10.1315/mjms2020.27.2.4.

[5] Nan, Q.Y., Piao, S.G., Jin, J.Z., Chung, B.H., Yang, C.W., & Li, C. (2024) Pathogenesis and management of renal fibrosis induced by unilateral ureteral obstruction. *Kidney Research and Clinical Practice, 43(5)*,586–99. https://doi.org/10.23876/j.krcp.23.156.

[6] Andrade-Oliveira, V., Foresto-Neto, O., Watanabe, I.K.M., Zatz, R., & Câmara, N.O.S. (2019) Inflammation in renal diseases: New and old players. *Frontiers in Pharmacology, 10(10)*,1–19. https://doi.org/10.3389/fphar.2019.01192.

[7] Olufunmilayo, E.O., Gerke-Duncan, M.B., & Holsinger, R.M.D. (2023) Oxidative stress and antioxidants in neurodegenerative disorders. *Antioxidants, 12(2)*,1–30. https://doi.org/10.3390/antiox12020517.

[8] Khater, Y., Barakat, N., Shokeir, A., Samy, A., & Karrouf, G. (2025) Renal fibrosis progression following partial unilateral ureteral obstruction: Mechanisms and therapeutic insights. *World Journal of Urology, 43(1)*. https://doi.org/10.1007/s00345-025-05580-x.

[9] Niculae, A., Gherghina, M.E., Peride, I., Tiglis, M., Nechita, A.M., & Checherita, I.A. (2023) Pathway from acute kidney injury to chronic kidney disease: Molecules involved in renal fibrosis. *International Journal of Molecular Sciences, 24(18)*. https://doi.org/10.3390/ijms241814019.

[10] Sulaiman Alief, G., & Sangging, P.R.A. (2024) Malondialdehyde (MDA) sebagai marker stres oksidatif berbagai penyakit. *Medical Profession Journal of Lampung, 14(2)*,321–325.

[11] Hojs, N.V., Bevc, S., Ekart, R., & Hojs, R. (2020) Oxidative stress markers in chronic kidney disease with emphasis on diabetic nephropathy. *Antioxidants. 9(10)*,1–22. https://doi.org/10.3390/antiox9100925.

[12] Patera, F., Gatticchi, L., Cellini, B., Chiasserini, D., & Reboldi, G. (2024) Kidney fibrosis and oxidative stress: From molecular pathways to new pharmacological opportunities. *Biomolecules. 14(1)*, 1–16. https://doi.org/10.3390/biom14010137.

[13] Wang, L., Lan, W., & Chen, D. (2024) Blueberry (Vaccinium spp.) anthocyanins and their functions, stability, bioavailability, and applications. *Foods, 13(17)*, 2851.

[14] Chis, M.S., Negrus, C., Colis, A., Borsai, O., Negrean, & Oana-Raluca, N. (2024) Bilberries vs . blueberries : A comprehensive review. *Horticulturae, 10(12)*, 1–26. https://doi.org/10.3390/horticulturae10121343.

[15] Ćujić, N., Šavikin. K., Janković, T., Pljevljakušić, D., Zdunić, G., & Ibrić, S. (2016) Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food Chemistry,194*, 135–42. https://doi.org/10.1016/j.foodchem.2015.08.008.

[16] Hesketh, E.E., Vernon, M.A., Ding, P., Clay, S., Borthwick, G., Conway, B., & Hughes, J. (2014) A murine model of irreversible and reversible unilateral ureteric obstruction. *Journal of Visualized Experiments, (94)*, 1–6. https://doi.org/10.3791/52559.

[17] Fauzi, A., Titisari, N., & Paramanandi, D.A. (2020) Role of anthocyanin-blueberry extract in the histopathological improvement of renal fibrosis-unilateral ureteral obstruction (Uuo) model. *Veterinary Practice, 21(2)*, 467–71.

[18] Jang, K.Y., Sung, M.J., Zhang, X., Park, S.K., & Kim, W. (2009) Mast cells decrease renal fibrosis in unilateral ureteral obstruction. *Kidney International,75(10)*, 1031–8. https://doi.org/10.1038/ki.2009.1.

[19] Permata, F.S., & Febrianto, A. (2019) Salep ekstrak kulit buah naga (Hylicereus costaricensis) menurunkan ekspresi

interleukin- 2) dan jumlah sel radang mononuklear terhadap luka terbuka di kulit tikus strain wistar the ointment of peel dragon fruit *(Hylicereus costaricensis)* extract dec. *Veterinary Biomedical and Clinical Journal, 1(2)*, 24–34.

[20] Fauziah, P.N., Maskoen, A.M., Yuliati, T., & Widiarsih, E. (2018) Optimized steps in determination of malondialdehyde (MDA) standards on diagnostic of lipid peroxidation. *Padjadjaran Journal of Dentistry, 30(2)*,136. https://doi.org/ 10.24198/pjd.vol30no2.18329.

[21] Hosseinian, S., Rad, A.K., Bideskan, A.E., Soukhtanloo, M., Sadeghnia, H., Shafei, M.N., Motejadded, F., Mohebbati, R., Shahraki, S., & Beheshti, F. (2017) Thymoquinone ameliorates renal damage in unilateral ureteral obstruction in rats. *Pharmacology Reports, 69(4)*,648–57. https://doi.org/10.1016/j.pharep.2017.03.00

[22] Hassan, N.M.E., Shehatou, G.S.G., Kenawy, H.I., & Said, E. (2021) Dasatinib mitigates renal fibrosis in a rat model of UUO via inhibition of Src/STAT-3/NF-κB signaling. *Environmental Toxicolology and Pharmacology, 84*(October 2020),103625. https://doi.org/10.1016/j.etap.2021.103625.

[23] Panizo, S., Martínez-Arias, L., Alonso-Montes, C., Cannata, P., Martín-Carro, B., Fernández-Martín, J.L., Naves-Díaz, M., Carrillo-López, N., & Cannata-Andía, J.B. (2021) Fibrosis in chronic kidney disease: Pathogenesis and consequences. *International Journal of Molecular Sciences, 22(1)*, 1–19. https://doi.org/ 10.3390/ijms2201 0408.

[24] Nørregaard, R., Mutsaers, H.A.M., Frøkiær, J., & Kwon, T.H. (2023) Obstructive nephropathy and molecular pathophysiology of renal interstitial fibrosis. *Physiological Reviews, 103(4)*, 2827–72. https://doi.org/10. 1152/ physrev.00027.2022.

[25] Zhang, M., Guo, Y., Fu, H., Hu, S., Pan, J., Wang, Y., Cheng, J., Song, J., Yu, Q., Zhang, S., Xu, J.F., Pei, G., Xiang, X., Yang, P., & Wang, C.Y. (2015) Chop deficiency prevents UUO-induced renal fibrosis by attenuating fibrotic signals originated from Hmgb1/TLR4/NFκB/IL-1ß signaling. Cell *Death and Disease, 6,1–11.* https://doi.org/10.1038/cddis.2015.206.

[26] Ham, O., Jin, W., Lei, L., Huang, H.H., Tsuji, K., Huang, M., Roh, J., Rosenzweig, A., & Lu, H.A.J. (2018) Pathological cardiac remodeling occurs early in CKD mice from unilateral urinary obstruction, and is attenuated by Enalapril. *Scientific Reports 8(1),* 1–17. https://doi.org/10.1038/s41598-018-34216-x.

[27] Bonventre, J.V., & Yang, L. (2011) Cellular pathophysiology of ischemic acute kidney injury. *Journal of Clinical Investigation, 121(11)*, 4210–21. https://doi.org/10.1172/ JCI45161.

[28] Aranda-Rivera, A.K., Cruz-Gregorio, A., Aparicio-Trejo, O.E., Ortega-Lozano, A.J., & Pedraza-Chaverri, J. (2021) Redox signaling pathways in unilateral ureteral obstruction (UUO)-induced renal fibrosis. *Free Radical Biology and Medicine, 172,* 65–81. https://doi.org/10.1016/j.freeradbiomed.2021. 05.034.

[29] Pereira, S,R,, Pereira, R., Figueiredo, I., Freitas, V., Dinis, T.C.P., & Almeida, L.M. (2017) Comparison of anti-inflammatory activities of an anthocyanin-rich fraction from Portuguese blueberries *(Vaccinium corymbosum L.)* and 5-aminosalicylic acid in a TNBS-induced colitis rat model. *PLoS One, 12(3)*, e0174116. https://doi.org/10. 1371/journal.pone.0174116.

[30] Pyrillou, K., Burzynski, L.C., & Clarke, M.C.H. (2020) Alternative pathways of IL-1 activation, and its role in health and disease. *Frontiers in Immunology, 11(12),* 1–19. https://doi.org/10.3389/fimmu.2020. 613170.

[31] Cavalli, G., Colafrancesco, S., Emmi, G., Imazio, M., Lopalco, G., Maggio, M.C., Sota, J., & Dinarello C.A. (2021) Interleukin 1α: A comprehensive review on the role of IL-1α in the pathogenesis and treatment of autoimmune and inflammatory diseases.

Autoimmunity Reviews 20(3). ttps://doi.org/10.1016/j.autrev.2021.102763.

[32] Pap, N., Fidelis, M., Azevedo, L., do Carmo, M.A.V., Wang, D., Mocan, A., Pereira, E.P.R., Xavier-Santos, D., Sant'Ana, A.S., Yang, B., & Granato, D. (2021) Berry polyphenols and human health: Evidence of antioxidant, anti-inflammatory, microbiota modulation, and cell-protecting effects. *Current Opinion in Food Science, 42,* 167–86. https://doi.org/10.1016/j.cofs.2021.06.003.

[33] Kowalczyk, T., Muskała, M., Merecz-Sadowska, A., Sikora, J., Picot, L., & Sitarek, P. (2024) Anti-inflammatory and anticancer effects of anthocyanins in in vitro and in vivo studies. *Antioxidants, 13(9).* https://doi.org/ 10.3390/antiox13091143.

[34] Abais, J.M., Xia, M., Zhang, Y., Boini, K.M., & Li, P.L. (2015) Redox regulation of NLRP3 inflammasomes: ROS as trigger or effector? *Antioxidants & Redox Signaling, 22(13),* 1111–29. https://doi.org/10.1089/ars.2014. 5994.

[35] Alsuwayt, B. (2025) ROS-Mediated NLRP3 inflammasome activation in the progression of hypertensive nephropathy and its therapeutic interventions. *Journal of Young Pharmacists [Internet]17(1)*: 70–8. https://doi.org/10.5530/jyp.20251476.

[36] Wang, H., Wu, J., Ma, L., Bai, Y., & Liu, J. (2023) The role of interleukin -1 family in fibrotic diseases. Cytokine,165(3). https://doi.org/10.1016/j.cyto.2023.156161.

[37] Gyurászová, M., Gurecká, R., Bábíčková, J., & Tóthová, Ľ. (2020) Oxidative stress in the pathophysiology of kidney disease: Implications for noninvasive monitoring and identification of biomarkers. *Oxidative Medicine and Cellular Longevity, 2020*, 5478708. 10.1155/2020/5478708.

[38] Bahramibanan, F., Rad, M.V., Ranjbar, A., Karbasi, A., & Abbasifard, A. (2025) Comparison of oxidative stress status in the kidney tissue of male rats treated with paraquat and nanoparaquat. *Scientific Reports,15(1),* 1–10. https://doi.org/10.1038/ s41598-024-83156-2.

[39] Sadowska-Bartosz, I., & Bartosz, G. (2024) Antioxidant activity of anthocyanins and anthocyanidins: A critical review. *International Journal of Molecular Sciences, 25(22).* https://doi.org/10.3390/ ijms252212 001.

[40] Tena, N., Martín, J., & Asuero, A.G. (2020) State of the art of anthocyanins: Antioxidant activity, sources, bioavailability, and therapeutic effect in human health. *Antioxidants, 9(5).* https://doi.org/10.3390/ antiox9050451.

[41] Gui, H., Dai, J., Tian, J., Jiang, Q., Zhang, Y., Ren, G., Song, B., Wang, M., Saiwaidoula, M., Dong, W., & Li, B. (2023) The isolation of anthocyanin monomers from blueberry pomace and their radical-scavenging mechanisms in DFT study. *Food Chemistry, 418(March)*,135872. https://doi.org/10.1016/ j.foodchem.2023.135872.

[42] Merecz-Sadowska, A., Sitarek, P., Kowalczyk, T., Zajdel, K., Jęcek, M., Nowak, P., & Zajdel, R. (2023) Food anthocyanins: Malvidin and its glycosides as promising antioxidant and anti-inflammatory agents with potential health benefits. *Nutrients, 15*(13). https://doi.org/10.3390/nu15133016.

[43] Bendokas, V., Stanys, V., Mažeikienė, I., Trumbeckaite, S., Baniene, R., & Liobikas, J. (2020) Anthocyanins: From the field to the antioxidants in the body. *Antioxidants, 9(9)*, 1–16. https://doi.org/10.3390/antiox909081.

[44] Liu, Y. (2011) Cellular and molecular mechanisms of renal fibrosis. *Nature Reviews Nephrology. 7(12)*, 684–96. https://doi.org/10.1038/nrneph.2011.149.

[45] Walraven, M., & Hinz, B. (2018) Therapeutic approaches to control tissue repair and fibrosis: Extracellular matrix as a game changer. *Matrix Biology, 71–72* (2017), 205–24. https://doi.org/10.1016/j.matbio.2018.02. 020.