Original Article

Blueberry Extract Mitigates Inflammation and Fibrosis by Suppressing NF-κB and TGF-β in Kidney Nephropathy

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ABSTRACT

Background: Chronic kidney disease (CKD) is a global health issue marked by progressive inflammation and fibrosis. The unilateral ureteral obstruction (UUO) model is commonly used to study obstructive nephropathy, yet effective treatments targeting its underlying mechanisms are limited.

Objectives: This study aimed to evaluate the renoprotective effects of blueberry (*Vaccinium corymbosum*) extract on inflammation and fibrosis markers in a murine model of UUO.

Methods: Male Swiss Webster mice were divided into five groups: sham, UUO day 7, UUO + blueberry day 7, UUO day 14, and UUO + blueberry day 14. Blueberry extract (1500 mg/kg BW) was administered daily via oral gavage. UUO was induced by surgical ligation of the right ureter under anesthesia. Kidney tissues were examined macroscopically and histologically. Blood urea nitrogen (BUN) and creatinine levels, glomerulonephritis scores, and immunohistochemical expression of NF-κB and TGF-β were assessed.

Results: UUO caused right kidney enlargement by day 7 and severe hydronephrosis by day 14, along with significantly higher glomerulonephritis scores and increased expression of NF-κB and TGF-β ($P<0.05$ vs the SC group). Blueberry extract administered for 7 or 14 days improved kidney structure, lowered glomerulonephritis scores ($P=0.53$ and $P=0.87$, respectively), and significantly reduced NF-κB and TGF-β levels ($P<0.05$ vs the UUO group). BUN and creatinine levels were slightly higher in the UUO groups but showed no significant difference ($P>0.05$ vs the SC group), with lower values in the treated groups.

Conclusion: These findings suggest that blueberry extract may protect against UUO-induced obstructive nephropathy by reducing inflammation and fibrosis.

Keywords: Blueberry, Renal fibrosis, NF-κB, TGF-β, Hydronephrosis

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Introduction

Chronic kidney disease (CKD) has become a major public health issue worldwide, with persistent inflammation now recognised as a key driving force in its pathogenesis. This state of chronic inflammation not only induces progressive injury of the kidney but also increases the risk of the incidence of death and cardiovascular events (Kadatane et al. 2023). Renal fibrosis, manifested by abnormal accumulation of extracellular matrix (ECM) components leading to renal architectural disruption and irreversible loss of kidney function, is one of the most important characteristics of advancing CKD (Reiss et al., 2024). Fibrosis is not only an effect of CKD but also an instigator, accelerating its progression to end-stage renal disease (ESRD), which is a lethal step associated with high morbidity and mortality (Yuan et al., 2022). For the most part, as a result of its asymptomatic nature, CKD is not usually diagnosed until it is too late for any therapeutic approaches to be effective (Mihai et al., 2018). In addition, nutritional approaches have been gaining growing interest; a recent meta-analysis has reported an association between healthy dietary patterns and a decreased risk of CKD and albuminuria, reflecting the preventive effect of nutritional factors on the maintenance of renal function (Bach et al., 2019). The role of bioactive compounds in modulating molecular pathways of disease has been widely studied in various experimental models. For instance, curcumin has been reported to influence GLP-1R expression in the liver tissue of diabetic rats (Uğran & Koral Taşçı, 2024), and surgical interventions have been shown to transiently affect renal biochemical parameters in dogs (Yousefi Ghadikolaei et al., 2024). These findings underscore the importance of both dietary and procedural interventions in modulating biochemical and histopathological outcomes, thereby supporting the rationale for investigating natural antioxidants, such as blueberry extract, in the context of kidney disease.

The traditional pharmacological therapies for obstructive nephropathy-associated fibrosis remain unsatisfactory due to their limited efficacy in reversing fibrotic changes and the potential harm associated with chronic administration. This has fueled increasing interest in investigating plant-based anti-inflammatory and antioxidant compounds as adjuvants (Chen et al., 2019). Among these, blueberries (*Vaccinium* spp.) are notable candidates due to their richness in anthocyanins—natural polyphenols with high antioxidant properties (Khoo et al., 2017). Anthocyanins have been shown to scavenge reactive oxygen species (ROS), downregulate pro-inflammatory cytokines, and mitigate cellular damage in various disease models (Khan et al., 2022; Spormann et al., 2008). Black currants and blueberries have higher levels of natural antioxidants than other small berries, which is valuable in therapeutic nutrition (Zorzi et al., 2020). Meanwhile, nuclear factor-kappa B (NF-κB) and transforming growth factor-beta (TGF-β) are key signalling molecules involved in inflammation and fibrosis (Oh et al., 2023). Nevertheless, there is still limited knowledge on whether blueberries modulate important fibrotic and inflammatory features, including NF-κB and TGF-β, in models of chronic renal damage. To address this gap, we suggest investigating the potential protective effects of anthocyanin-rich blueberry extract as a natural intervention targeted at inflammation and fibrosis pathways in obstructive kidney disease.

The central problem statement of this study lies in the urgent need to identify effective and safe strategies to counteract fibrosis and inflammation in obstructive nephropathy, where synthetic drugs fall short. Based on the known bioactive properties of blueberries, we hypothesized that blueberry extract can downregulate NF-κB and TGF-β expression, thus alleviating the inflammatory and fibrotic burden in unilateral ureteral obstruction (UUO)-induced kidney fibrosis. This research contributes to the field by providing mechanistic insights into the molecular effects of blueberry anthocyanins on obstructed kidney pathology. Furthermore, it offers a potential nutraceutical-based therapeutic approach that could complement conventional treatments and delay the onset of ESRD. By integrating molecular, histological, and biochemical data, the study establishes a foundation for future translational applications of natural antioxidants in renal medicine. This study aimed to investigate the effect of blueberry extract on NF-κB and TGF-β expression in a murine model of obstructive kidney fibrosis induced by UUO. The intervention's impact was evaluated through immunohistochemical analysis of kidney tissues to quantify the expression of NF-κB and TGF-β, along with additional assessments of kidney morphology, inflammation, and fibrosis parameters. This comprehensive methodological approach allows for a robust evaluation of the therapeutic potential of blueberry extract in treating renal fibrosis.

Materials and Methods

Study period and location

This study was conducted from September to December 2021 at the Animal House Facility of the Faculty of Medicine, University of Brawijaya, Malang, Indonesia.

The blueberries were extracted in the Materia Medica Laboratory, Ministry of Health, Batu, East Java, Indonesia. The serum biochemical assay was analyzed at the clinical pathology laboratory, while immunohistological analysis was conducted in the biomedical laboratory at the Faculty of Medicine, University of Brawijaya.

Preparation of blueberry extract

Fresh blueberries (*Vaccinium corymbosum*) were washed and homogenized using a blender. Five hundred grams of the resulting mash were measured, followed by the addition of 1,500 mL of 70% ethanol. The solution was homogenized at a rate of 50 rpm and subsequently filtered using filter paper No. 1 (Whatman, UK). The filtrate was concentrated using a rotating evaporator (Rotavapor, Buchi, Switzerland) at 40 ˚C. The extract obtained was subsequently stored in a refrigerator at 4 ˚C for further use. Phytochemical screening of the blueberry extract confirmed the presence of anthocyanins, as indicated by the characteristic color reaction in acidic and alkaline conditions. However, no quantitative standardization of anthocyanin content was performed in this study.

Experimental animals

Twenty-five male mice (Mus musculus) of the Swiss Webster strain, with body weights ranging around 27±3 g, were obtained from the animal laboratory at the Faculty of Mathematics and Life Sciences, University of Brawijaya, and housed at the animal experiment laboratory at the Faculty of Medicine, University of Brawijaya. Upon arrival, the animals were acclimatized for a week and fed a standard commercial rodent diet and plain water ad libitum. The experimental animals were divided into five groups: (1) a sham-operated control group (SC) that received saline without UUO; (2) a UUO control group evaluated on day 7 (UUO-7) and administered saline for 7 days; (3) a group treated with blueberry extract for 7 days following UUO (UUO-BB7, 1.500 mg/kg body weight); (4) a UUO control group assessed on day 14 (UUO-14), receiving saline for 14 days; and (5) a group treated with blueberry extract for 14 days post-UUO (UUO-BB14, 1500 mg/kg body weight). According to their group treatment, all treatments were given orally using oral gavage once daily in the morning. The dose of 1.500 mg/kg was selected based on our prior study on nephrotoxic rodents (Fauzi et al., 2021). Day 7 was chosen to represent the early inflammatory phase of UUO, whereas day 14 corresponded to the more established fibrotic phase, as described in previous UUO studies (Hesketh et al., 2014).

Induction of obstructive nephropathy

Obstructive nephropathy was induced using the UUO technique, as outlined by Hesketh et al., with minor modifications —a widely validated model that mimics the progression of renal interstitial fibrosis (Hesketh et al., 2014). Mice were anesthetized with ketamine (70 mg/kg body weight) and xylazine (15 mg/kg body weight), and the right flank area was shaved and disinfected. A 1.5 cm incision was made to expose the kidney and ureter, which was carefully dissected and ligated using 3/0 silk sutures. The kidney was then returned to the peritoneal cavity, and the incision was sutured in layers. Post-operative care included oral administration of ibuprofen (30 mg/kg body weight) as analgesia and daily monitoring for signs of distress.

Blood and organs collection

At the end of the study, mice were fasted overnight, and blood samples of approximately 1.5 mL were collected through cardiac puncture under anesthesia. The blood samples were inserted into plain red-top sterilized centrifuge tubes (BD Vacutainer®, USA). The serum was separated from the blood samples using a centrifuge (Eppendorf, Germany) at 4000 rpm for 15 min and stored at –20 ˚C until further analysis. Kidneys from each mouse were harvested following tissue perfusion with PBS. The mouse kidneys were cleansed of leftover blood using PBS. The organs were subsequently preserved in a 10% formalin buffer for histological analysis.

Determination of serum biochemical parameters in mice treated with blueberry extract

Serum was obtained from mice, and renal function was analyzed by determining the blood urea nitrogen (BUN) and creatinine levels. Following the manufacturer's instructions, BUN and creatinine levels were measured using a Pentra c400 semi-automatic chemical analyzer (Horiba, Tokyo, Japan).

The kidney haematoxylin and eosin staining and scoring

Initially, following fixation, the tissues were embedded in paraffin and sliced into thin sections, ranging from 4 to 8 µm, using a microtome (Leica, Germany). These sections were then placed on slides. The sections were then hydrated through a series of washes in graded alcohols, followed by staining with hematoxylin, which binds to nuclear material, producing a blue color. After rinsing, the sections were stained with eosin, which pro-

vides contrast by staining the cytoplasmic components pink. Finally, the prepared sections were dehydrated and cover-slipped for microscopic examination (Wang et al., 2023). Glomerulonephritis scoring was performed according to the standardized histopathological criteria described by Gibson-Corley et al. using a 0–3 semi-quantitative scale. Each glomerulus within a defined area was evaluated for necrosis levels, which were categorized semi-quantitatively as grade zero (no damage), one (little to no sclerosis or inflammation), two (some glomeruli show mild segmental sclerosis and inflammation), and three (most glomeruli are affected by widespread sclerosis and fibrosis) according to standardized scoring systems (Gibson-Corley et al., 2013). The percentage of abnormal glomeruli was then calculated and reported as the glomerulonephritis scoring (de Zoysa et al., 2024). Histological and immunohistochemical assessments were performed in a blinded manner by two independent observers, without knowledge of the group assignments, to ensure objective and unbiased evaluation of tissue morphology and marker expression.

Immunohistochemistry of NF-κB and TGF-β on kidney tissue

The embedded tissue section was deparaffinized and dehydrated, and antigen retrieval was performed for 5 min, followed by treatment with a peroxidase solvent with containing 5% H_2O_2 buffer for 15 min. Next, 5% bovine serum albumin was applied to the section for blocking for 30 min. The section was then incubated overnight at 4 ˚C with primary antibodies (1:250 NF-κB/p65, Santa Cruz, USA, or 1:250 TGF-β, Bioss, USA). The section was incubated with a biotinylated secondary antibody, N-Histofine Simple Stain-TM mouse (Nichirei, Japan), for 30 min and stained with diaminobenzidine (DAB) for 3 min. Slides were counterstained with hematoxylin for 3 min before being mounted with DPX mounting histology (Sigma, USA) and covered with a cover glass.

Semi-quantification of NF-κB and TGF-β expression

The expressions of NF-κB and TGF-β were observed under a 400x-magnification light microscope (Olympus, Tokyo, Japan). Slides were captured in five random fields of view in the cortical area and then quantified using ImageJ software, version 1.54p (NIH, USA). DAB brown area expression in the kidney tissue was identified as a positive area of expression.

Statistical analysis

All statistical analyses were performed using SPSS software, version 21.0 (IBM Corp., Armonk, NY, USA). The normality of the data was assessed using the Shapiro-Wilk test. Data are expressed as Mean±SD for normally distributed variables (e.g. NF-κB, TGF-β), and median (Q1–Q3) for non-normally distributed variables (e.g. BUN, creatinine, glomerulonephritis scores). One-way ANOVA followed by Tukey's post hoc test was used for normally distributed data. For non-parametric data, Kruskal-Wallis test was followed by the Mann-Whitney test with Bonferroni correction for pairwise comparisons. A $P<0.05$ was considered statistically significant.

Results

Blueberry extract maintains kidney anatomy in UUO-induced mice

Mice subjected to UUO exhibited notable anatomical alterations in the right kidney, with visible enlargement observed as early as the seventh day post-surgery (Figure 1A). By the 14th day, the pathological progression was marked by pronounced hydronephrosis, indicating severe urinary retention and renal structural damage. These morphological changes are indicative of progressive fibrosis and impaired renal function due to ureteral blockage. In contrast, mice treated with blueberry extract for both 7 and 14 days demonstrated significantly improved renal morphology.

Effect of blueberry extract on BUN and creatinine levels in UUO-induced mice

The data presented in Table 1 summarize the BUN and serum creatinine concentrations in Swiss Webster mice subjected to UUO with or without blueberry extract treatment for 7 and 14 days. These parameters are commonly used clinical indicators of renal function. Across all experimental groups, BUN levels ranged from 13.5 mg/dL in the sham control (SC) group to 17.25 mg/dL in the UUO-BB7 group. Although mice in the UUO-induced groups exhibited slightly elevated BUN levels compared to the control group, the differences were not statistically significant ($P>0.05$). Similarly, treatment with blueberry extract in both the 7-day (UUO-BB7: 17.25 mg/dL) and 14-day (UUO-BB14: 17.1 mg/dL) groups did not result in a significant reduction in BUN compared to their corresponding untreated UUO groups. In terms of serum creatinine, a slight increase was observed in the UUO groups (UUO-7: 0.4 mg/dL; UUO-14: 0.45 mg/dL) relative to the SC (0.3 mg/dL), indicating mild impairment in glomerular filtration. However, as with BUN, these differences did not reach statistical significance.

Table 1. Serum BUN and creatinine concentrations in UUO-induced Swiss Webster mice on days 7 and 14

Experimental Groups	Median (Q1-Q3)	
	BUN (mg/dL)	Creatinine (mg/dL)
SC (n=5)	13.5 (12.37-16.5)[a]	0.3 (0.3-0.37)[a]
UUO-7 (n=5)	16.25 (14.63-21.47)[a]	0.4 (0.4-0.4)[a]
UUO-BB7 (n=5)	17.25 (14.42-17.83)[a]	0.4 (0.4-0.4)[a]
UUO-14 (n=5)	15.25 (14.72-21.47)[a]	0.45 (0.32-0.5)[a]
UUO-BB14 (n=5)	17.10 (16.03-19.75)[a]	0.4 (0.32-0.4)[a]

Abbreviations: SC: Sham-operated control group; UUO-7: The UUO control group evaluated on day 7; UUO-BB7: A group treated with blueberry extract for 7 days following UUO; UUO-14: A UUO control group assessed on day 14; UUO-BB14: A group treated with blueberry extract for 14 days post-UUO.

[a]No significant differences between groups (P>0.05).

Blueberry extract reduced glomerulonephritis scoring and NF-κB and TGF-β expression levels in UUO-induced mice

Table 2 presents the glomerulonephritis scoring data, indicating that ureteral ligation induced significant glomerular damage in mice, as evidenced by increased scores in both UUO-7 (1.26) and UUO-14 (1.53) groups compared to the sham control group (0.0). Treatment with blueberry extract markedly reduced the severity of glomerulonephritis, with scores significantly lower in the UUO-BB7 (0.53) and UUO-BB14 (0.87) (P<0.05) groups compared to their respective untreated UUO groups. On the other hand, the expression levels of NF-κB and TGF-β, two critical mediators of inflammation and fibrosis, were assessed. The SC exhibited the lowest expression levels for both NF-κB (19.98±3.13) and TGF-β (22.68±2.9), representing normal baseline levels. In contrast, UUO significantly upregulated the expression of both markers. At day 7, NF-κB expression in the UUO-7 group rose to 55.86±3.77, and TGF-β increased to 58.99±2.45—both significantly higher than the controls (P<0.05). These levels increased even further in the UUO-14 group, with NF-κB reaching 67.56±4.29 and TGF-β at 82.18±2.9, indicating progressive inflammatory and fibrotic responses due to prolonged obstruction (P<0.05). Importantly, blueberry extract treatment resulted in significant reductions in both NF-κB and TGF-β expression levels compared to untreated UUO groups. The UUO-BB7 group showed a marked decrease in NF-κB levels to 28.85±5.45 and in TGF-β levels to 38.70±7.3, with NF-κB levels comparable to those of the sham group (P<0.05). Similarly, the UUO-BB14 group exhibited significantly lower expression of NF-κB (43.15±6.41) and TGF-β (52.52±13.44) compared to the UUO-14 group, indicating a therapeutic effect despite ongoing fibrosis (P<0.05).

Table 2. Glomerulonephritis scoring and NF-κB and TGF-β expression levels in the kidney of UUO-induced Swiss Webster mice on days 7 and 14 (n=5)

Experimental Groups	Median (Q1-Q3)	Mean±SD	
	Glomerulonephritis Scoring	NF-κB Expression	TGF-β Expression
SC (n=5)	0.0 (0.0-0.0)[a]	19.98±3.13[a]	22.68±2.9[a]
UUO-7 (n=5)	1.26 (1.20-1.33)[c]	55.86±3.77[c]	58.99±2.45[c]
UUO-BB7 (n=5)	0.53 (0.48-0.68)[ab]	28.85±5.45[a]	38.7±7.3[ab]
UUO-14 (n=5)	1.53 (1.53-1.58)[c]	67.56±4.29[d]	82.18±2.9[d]
UUO-BB14 (n=5)	0.87 (0.82-0.92)[b]	43.15±6.41[b]	52.52±13.44[bc]

Abbreviations: SC: Sham-operated control group; UUO-7: The UUO control group evaluated on day 7; UUO-BB7: A group treated with blueberry extract for 7 days following UUO; UUO-14: A UUO control group assessed on day 14; UUO-BB14: A group treated with blueberry extract for 14 days post-UUO. Note: Different notations indicate significant differences (P<0.05).

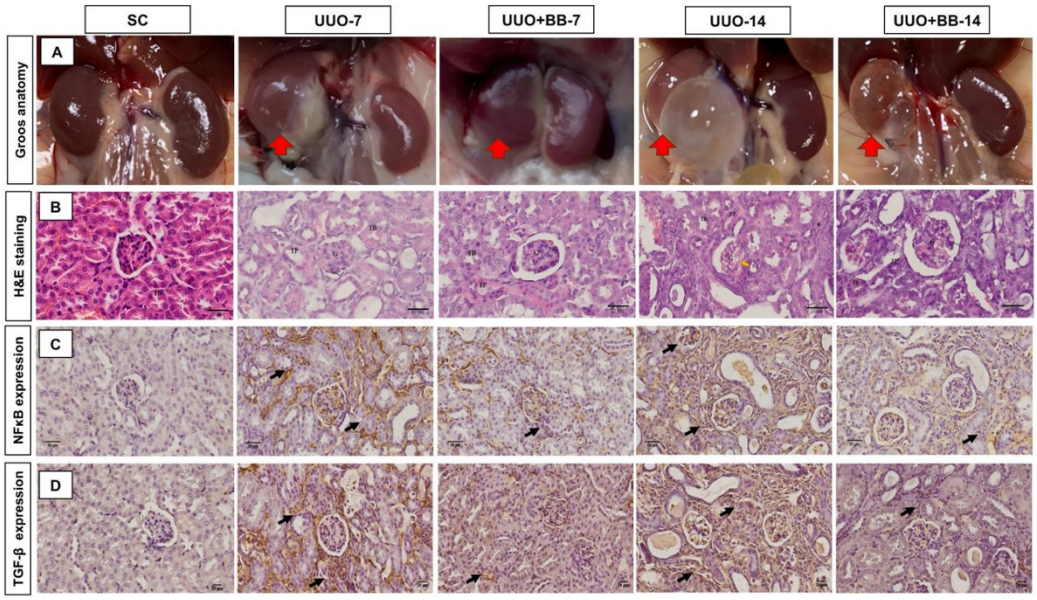

Figure 1. Effects of blueberry extract on kidney morphology, histopathology, and NF-κB/TGF-β expression in UUO mice

A) The gross architecture of the ligated ureteral kidney (red arrow) exhibited an increase in volume with hydronephrosis on days 7 and 14. Blueberry treatment improved kidney anatomy by maintaining the kidney volume and lessening hydronephrosis; B) H&E staining presents mild to severe tubular and glomerular necrosis in the mice's kidneys on days 7 and 14 (yellow arrow). Nevertheless, the blueberry treatment improves kidney structure by lessening the necrosis in the tubular and glomerular regions; C) NF-κB expression in the kidney; the ligated ureteral kidney demonstrated elevated NF-κB expression in the tubules on days 7 and 14. Meanwhile, blueberry administration reduced NF-κB expression in the tubules on days 7 and 14; D) TGF-β expression in the kidney; the ligated ureteral kidney displayed increased TGF-β expression in interstitial tubules on days 7 and 14. In the meantime, blueberry administration decreased TGF-β expression in the interstitial tubules on days 7 and 14

Abbreviations: G: Glomerulus; TD: Distal tubule; TP: Proximal tubule.

Note: The black arrow indicates the diaminobenzidine-positive NF-κB and TGF-β expression area (magnification: 400x; scale bar: 20 μm).

Discussion

This study demonstrated that blueberry extract exerted renoprotective effects in mice subjected to UUO, as evidenced by reduced glomerular injury, lowered NF-κB and TGF-β expression, and improved kidney morphology. In the gross anatomy observation, apparent dilation and hydronephrosis in the UUO kidneys were observed, especially at day 14 of UUO (Figure 1A), typical of water retention and higher hydrostatic pressure (Hesketh et al., 2014). Conversely, treatment with blueberry extract on days 7 and 14 largely prevented these pathological alterations, suggesting that blueberry extract may help maintain the structure of the kidney under progressive injury. Interestingly, the traditional markers for renal function, such as BUN and creatinine, were only slightly elevated and not significantly different between the groups. The absence of substantial changes in BUN and creatinine levels may be attributed to the small sample size, model-specific characteristics, and inherent variability in biochemical responses, as demonstrated in previous studies on various disease models or interventions (Salman Jasim et al., 2022; Sulaiman et al., 2022). Moreover, the UUO model, due to its unilateral nature, permits compensation by the contralateral kidney, thereby masking systemic changes (Becker & Hewitson, 2013; Ho et al., 2021).

These results provide evidence of gross histological damage and, in the early stages of obstruction, lead to a functioning renal system with minimal systemic function, thus underlining the need for more sensitive biomarkers. However, blueberry-treated animals showed a tendency toward lower BUN and creatinine levels, indicating the potential for nephroprotection that might be more evident in more extended regimens. This finding is consistent with previous studies, suggesting that dietary polyphenols may delay the loss of function in kidney diseases by attenuating cellular stress and local inflammation (Ashkar et al., 2022).

Untreated UUO mice displayed marked glomerulonephritis and tubular necrosis on histopathological examination. A significant reduction in these structural alterations was observed in mice receiving blueberry extract, particularly in the 7-day treated group, which exhibited a significantly lower glomerulonephritis score. This observation suggests that blueberry extract offers protection not only in gross anatomy but also at the glomerular level, potentially through the reduction of inflammation and oxidative stress at the cellular level. This histological protection aligns with findings from other researchers who have examined polyphenol-rich extracts of various berries. For instance, in models of diabetic nephropathy and toxin-induced injury, cranberries and blackcurrants have been reported to demonstrate renoprotective activity by reducing histological damage and enhancing antioxidant capacity (Chen et al., 2019). Anthocyanins in blueberry, specifically delphinidin, cyanidin, and malvidin, have been extensively documented for their anti-apoptotic and anti-necrotic effects on kidney tissues (Lee et al., 2014; Veberic et al., 2015).

NF-κB has a central role in inflammation, mediating cytokine production, leukocyte trafficking, and tissue injury. Consistently, NF-κB levels were found to be significantly upregulated in the UUO renal tissues by day 7 and even more evidently by day 14, indicating continuous inflammation and immune activation in the kidney. Interestingly, blueberry extract supplementation greatly decreased the expression of NF-κB, particularly on day 7, bringing its levels close to those of the sham group. This interdiction suggests that compounds in blueberries impair dynamic pathways of inflammation. Anthocyanins possess strong antioxidant properties, which can reduce oxidative stress levels, inhibit NF-κB activation in renal tissues, and subsequently lead to a reduction in inflammatory mediators, thereby ameliorating kidney disease (Chen & Meng, 2022). IκB degradation, which impedes the translocation of NF-κB to the nucleus and thus the expression of TNF-α, a pro-inflammatory gene, can be suppressed by anthocyanins, the primary polyphenols found in blueberries (Hou et al., 2005; Serra et al., 2013). A recent study demonstrated that anthocyanin-enriched mulberry extracts effectively alleviated kidney inflammation in diabetic rats by inhibiting the NF-κB/NLRP3 pathway (Wang et al., 2023). Similarly, pterostilbene (a stilbene found in blueberries) attenuated kidney inflammation by altering the TLR4/NF-κB pathway (Feng et al., 2020). Thus, the results of the present study align with the existing literature and provide further evidence for the anti-inflammatory role of anthocyanins in renal pathologies.

TGF-β is a master regulator of fibrosis that stimulates ECM accumulation, myofibroblast activation, and tissue scarring through the Smad signalling pathway (Isaka, 2018). In the UUO model, the expression of TGF-β increased in the untreated kidneys, particularly at 14 days, suggesting its implication in the still-active fibrotic processes (Kim et al., 2016). The results of the present study showed that TGF-β expression decreased in the mice treated with blueberry extract, especially in the early intervention group (UUO-BB7). Blueberry may modulate TGF-β signaling predominantly by attenuating oxidative stress and consequently reducing inflammation (Felgus-Lavefve et al., 2022). This discovery is significant as it demonstrates that blueberry extract is antifibrotic, likely by attenuating oxidative stress and inflammatory stimuli that reduce TGF-β production (Baba et al., 2016). Moreover, a study showed that blueberry juice facilitated decreased lung and kidney fibrosis by inhibiting the TGF-β1/Smad3 pathway (Li et al., 2022). Another study demonstrated that blueberries possess multi-target antifibrotic actions, downregulating hepatic/renal RNA levels of TGF-β, PDGF, and FGF2 (Ashique et al., 2024). Such results strongly support the idea that blueberry extract works upstream within the fibrotic signalling pathway, dampening or halting the course of renal fibrosis.

One of the more interesting aspects of this study is the comparison of the 7-day versus 14-day treatment. The 7-day treatment not only reduced inflammatory and fibrotic markers as effectively as the 14-day treatment, but it also performed better, showing decreased glomerulonephritis scores and exhibiting more normalized NF-κB and TGF-β expression levels (Xianyuan et al., 2019). This finding indicates a sensitive period for therapeutic intervention, particularly when treated during the early stages of injury with blueberry extract (Nan et al., 2024). In addition, the results demonstrated that BUN and creatinine are relatively insensitive to early or localized kidney injury, whereas histological examination and molecular profiling can detect subtle, site-specific damage and early inflammatory or fibrotic responses before overt loss of function occurs. NF-κB and TGF-β are activated rapidly in response to injury, making them more responsive indicators of early treatment effects (Ezzat et al., 2025). This observation is also clinically relevant and emphasizes the importance of early diagnosis and dietary or pharmacological therapy in obstructive kidney disease. This is consistent with the report, finding that early antioxidant treatment in CKD models had a more substantial effect on stopping fibrosis than late-phase treatment (Yuan et al., 2022). The study fills the gap between dietary interventions and molecular pathophysiology, suggesting the novel potential of functional

foods or nutraceuticals in nephrology. During a time when plant-based, toxin-free remedies are becoming increasingly desirable, the results significantly support the use of dietary anthocyanins as a potential add-on therapy in the management of CKD (Khan et al., 2022; Reiss et al., 2024).

The study on the therapeutic potential of blueberry extract in mitigating kidney fibrosis has several limitations. The short treatment duration (7 and 14 days) may not fully capture its effects. The use of a UUO model limits the translation of systemic renal function outcomes. The study only used male mice, leaving sex-related differences unexplored. Direct functional assessments, such as urine output, proteinuria, GFR, and tubular function, were not included. Hence, future studies should include bilateral obstruction models, extended treatment durations, female subjects, and functional renal endpoints to validate and extend the findings.

Conclusion

Blueberry extract effectively mitigated hydronephrosis, glomerular injury, inflammation, and fibrotic signaling by downregulating NF-κB and TGF-β expression levels in mice with obstructive nephropathy at both 7 and 14 days post-UUO. Greater improvement was observed in the 7-day treatment group, highlighting the therapeutic value of early intervention. Despite the promising outcomes, this study is limited by its short duration, absence of direct renal function measurements, and the use of a unilateral model. Future research should investigate longer treatment regimens, female subjects, and functional parameters using chronic or bilateral obstruction models to further validate the nephroprotective potential of blueberry extract.

Ethical Considerations

Compliance with ethical guidelines

All experimental protocols employed in this work received approval from the Institutional Animal Care and Use Committee (IACUC) at the University of Brawijaya, Malang, Indonesia (Code: 045.KEP-UB).

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Authors' contributions

Conceptualization and writing the original draft: Ahmad Fauzi; Resources: Ahmad Fauzi, Nurina Titisari and Dini Agusti Paramanandi; Experiments and data curation: Sabila Madhani, Rizky Krisnanda Sinaga, and Toni Aditya Prayoga: Formal analysis: Ahmad Fauzi, Sabila Madhani, Rizky Krisnanda Sinaga, and Toni Aditya Prayoga; Validation, project administration, review and editing: Nurina Titisari and Dini Agusti Paramanandi; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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