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Inflammatory mediator responses of *Vaccinium corymbosum* extracts on the streptokinase induced acute glomerulonephritis in rats

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ABSTRACT

Blueberry (*Vaccinium corymbosum*) has many health benefits including anti-inflammatory and antioxidant activities. Glomerulonephritis is a commonly found kidney disease in companion animals that is characterized by glomerular proliferation and inflammation likes characteristics. The present study was carried out to evaluate the potential of blueberry against inflammatory response in the kidney of acute glomerulonephritis (AGN) in animal models. For this, twenty male Wistar rats were randomized into five groups i.e. A - E (n=4). Among these Group A has four healthy individuals administrated with aqua dest (negative control), group B individuals have streptokinase (6000IU/rat) induced acute glomerulonephritis rats treated with aqua dest (positive control) while group C-E has streptokinase (6000IU/rat) induced acute glomerulonephritis rats treated with different concentrations of blueberry extract (500, 1000, and 1500 mg/kg body weight) for 14 days, respectively. After 14 days, kidney samples were harvested for histology and immunohistochemistry examinations. One-way ANOVA followed by the Tukey test was used for statistical analysis ($P< 0.05$). The blueberry extract treated AGN rats showed a significantly decreased in IL-1beta expression and inflammatory cell numbers compared to negative and positive control rats and 1500 mg/kg of the blueberry extract was found as the optimal dose. Results of the study can be concluded that blueberry extract has a strong anti-inflammatory effect that could depress the inflammatory responses in acute glomerulonephritis rat animal models.

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1 Introduction

Glomerulonephritis is a kidney disorder that is characterized by an inflammation in the glomerular capillary and expanded permeability of the glomerular filtration barrier (GFB) (Medica et al. 2021). Post-streptococcal glomerulonephritis is the most common acute glomerulonephritis caused by β-hemolytic streptococcal nephritogenic strains group A (VanDeVoorde 2015). In animals, the incidence of acute post-streptococcal glomerulonephritis is common in dogs and cats, and about 55% of male dogs with an age of 4-8 years, and 75% of cats with an average age of 3-4 years are susceptible to this disease (Brown 2016).

Acute post-streptococcal glomerulonephritis occurs due to a complicated process that involves antibodies and antigens that interact in the blood and make immune complexes (Noris and Remuzzi 2013). Streptokinase is an extracellular protein derived from Streptococcus β-hemolytic (Shilpi et al. 2013) which converts free plasminogen into plasmin (an active enzyme) by breaking the peptide chain. This plasmin will bind to the blood vessels and degrade the fibrin polymer into small fragments, and this fibrin degradation increases vascular permeability (Brodsky and Nadasdy 2017). This process causes kidney damage and activates C3a and C5a complements (Keragala et al., 2018). This activation of the C3 and C5a complement increases the renal capillary permeability and increases IL-1β levels. IL-1β is a proinflammatory cytokine that is involved in fighting infection and functions as a neutrophil chemotactic factor, which will cause the release of neutrophils following the migration of eosinophils, basophils, and macrophages or inflammatory cell infiltration to the location of complimentary activation (Akdis et al. 2016). Treatment of glomerulonephritis can be performed by giving immunosuppressive drugs (cyclophosphamide or cyclosporine), diuretic drugs to elixir proteinuria, anti-inflammatory medications, and Angiotensin Converting Enzyme (ACE) inhibitors (for example, benazepril, enalapril, ramipril) (Brown, 2016). However, the side effect of the chemical drugs should be a concern and it could worsen the condition of kidney damage.

Blueberry (*Vaccinium corymbosum*) has 86% water content, 9.7% carbohydrates, 0.6% protein, and 0.4% fat. Moreover, blueberry has various antioxidant active ingredients such as ascorbic acid, polyphenols, and anthocyanins (Khoo et al. 2017). The anthocyanin contents in blueberries can be reached up to 495mg / 100g, including avidin, delphinidin, petunidin, cyanidin, and peonidin (Michalska and Łysiak 2015). Antioxidant contents protect body cells from free radicals by binding to free radicals to prevent inflammation in the cells (Alkhalf and Khalifa 2018). Ahmet et al. (2009) suggested that blueberry could protect the heart in ischemic conditions, and it could improve renal function (Nair et al. 2014). However, available information about how blueberry can inhibit the inflammation of acute glomerulonephritis is limited. Therefore, this study was carried out to determine the effect of blueberry extract on the proinflammatory cytokine of IL-1beta and total inflammatory cells in glomerulonephritis animal models induced by streptokinase.

2 Material and Methods

2.1 Animal experiment

In this study, six to eight weeks old (200±20g weight) twenty male Wistar rats were used as experimental animals. Before starting the experiment, ethical clearance was received from the university under approval no.1025-KEP-Universitas Brawijaya. These rats were randomly divided into 5 groups i.e. group "A" negative control (healthy individuals administrated with only aqua dest), group "B" positive control (AGN individuals treated by aqua dest), group "C" AGN individuals treated by 500 mg/kg of *Vaccinium sp.* extract, group "D" AGN individuals treated by 1000 mg/kg of *Vaccinium sp.* extract, and group "E" AGN individuals treated by 1500 mg/kg of *Vaccinium sp.* extract. All the individuals of Group B, C, D, and E were injected with streptokinase @ 6000 IU/rat to create an AGN animal model. After five weeks, all animals were euthanized and the kidney was collected for further analysis.

2.2 Streptokinase induced acute glomerulonephritis (AGN)

The dose of streptokinase given to experimental animals was 6000 IU/rat. Streptokinase vial (1,500,000 IU) (Fibrion, Dexa Medica, Indonesia) powder was diluted with aqua pro injection, afterward homogenized with vortex. At the end of the acclimatization period, streptokinase injection was given through the coccygeal vein. The streptokinase injection was given three times at five-day intervals on the 8th, 13th, and 18th days.

2.3 Blueberry Extract Therapy Administration

The Blueberry extract was prepared by the maceration method using 70% ethanol. Blueberry therapy was given to the C, D, and E groups for 14 days, gradually using oral gavage according to each group dose and each rat received 2 mL extract once a day.

2.4 Euthanization Method

Experimental animals were euthanized with 100mg/kg ketamine and 10mg/kg xylazine, followed by intracardial blood collection (Shomer et al. 2020). Rats were fixed in dorsal recumbency. Dissection was achieved by an abdomen incision, and subsequently, kidneys were collected. The kidney samples were washed with PBS and stored in an organ container filled with 10% formalin.

2.5 Immunohistochemistry for IL-1β

The whole sample was made from kidney tissues of all rats. Kidney tissues were fixed in original paraffin blocks and 4-µm

sections were cut from these blocks. The slide organs were washed with pH 7.4 PBS for 3 times for 5 minutes. Followed by the dripping of 2% H_2O_2 and the slide was left for 20 minutes. Afterward, it was soaked in 5% BSA in PBS for 30 minutes and subsequently washed 3 times for 5 minutes. The slide organs were fixed with primary antibodies (Anti rat IL-1β) overnight at 4°C and then washed with PBS. The slide organs were incubated with secondary antibodies labeled biotin (Anti Rabbit IgG biotin-labeled) for an hour at room temperature and washed with PBS. SA-HRP was dropped into a slide for 40 minutes and washed with PBS. Diaminobenzidine (DAB) and substrate solution were given and incubated for 10 minutes and counterstaining was performed using Mayer's hematoxylin for five minutes at 27 °C, and then the slides were washed with water, dried, and mounted using entellant.

2.6 Quantification of IL-1β expression

The *IL-1β* immunopositive cells were determined over at least five random histological fields under 400 x magnifications. The brown stained area on the slide would be calculated as a percentage (%) of the expression area using the ImmunoRatio image analysis software.

2.7 Inflammatory Cells Count

The histological examination was performed on kidney tissue samples with hematoxylin-eosin (HE) staining. Under the microscope, the kidney micrographs were evaluated by counting inflammatory cells over five visual fields, equalling one mm^2. The results counted as cells/mm^2 (Alzahrani et al. 2021).

2.8 Data Analysis

The data analysis was conducted quantitatively applying one-way ANOVA (analysis of variance) continued by Tukey's post hoc test using the SPSS software with a 95% confidential value.

3 Result and Discussion

3.1 Effect of Blueberry Extract on Interleukin 1 Beta Expression

To examine the influence of blueberry extract on AGN, IL-1β expression was measured as one of cytokine pro-inflammatory. The results of the present study revealed that streptokinase induced an inflammatory response in form of IL-1β, which can be marked as brown stained in all groups except the control

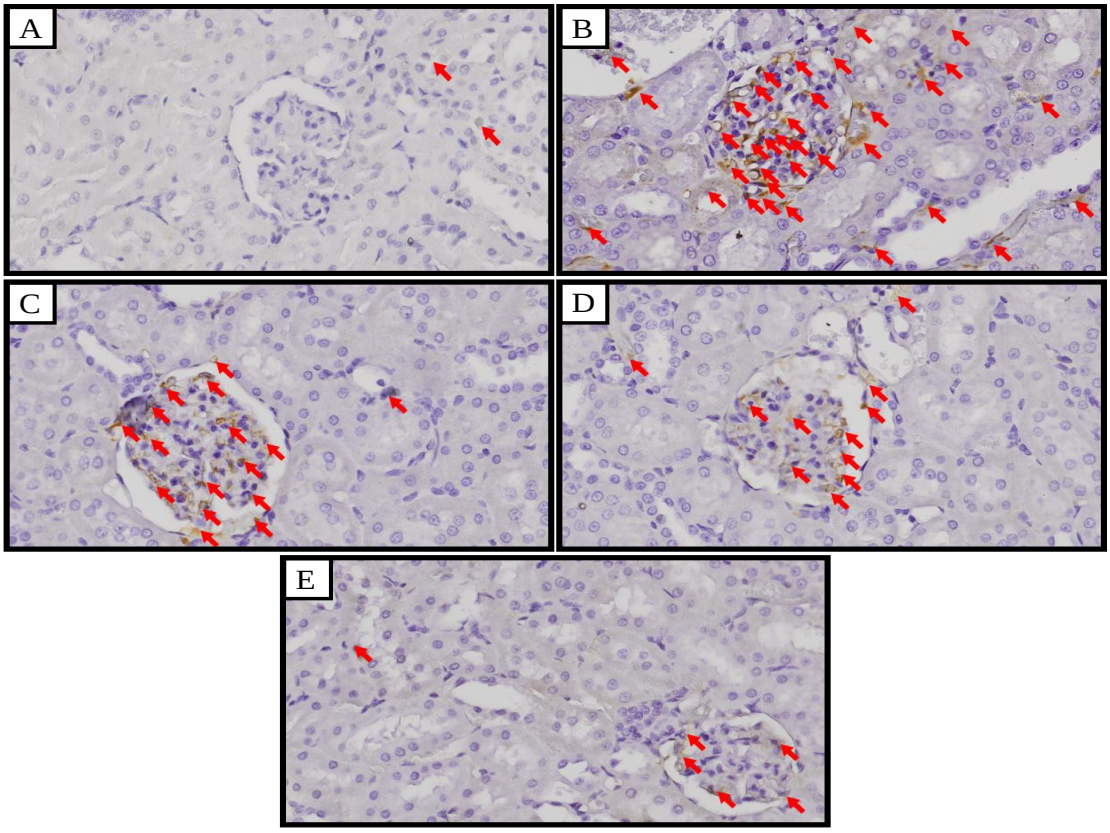

Figure 1 Blueberry therapy inhibit interleukin 1β (IL-1β) expression in the kidney of glomerulonephritis rat model (Immunohistochemistry: magnification of 400x), here A= Negative control; B= Positive control (glomerulonephritis/AGN); C= AGN with Blueberry extract dose 500mg/kgBW; D= AGN with Blueberry extract dose 1000mg/kgBW; E= AGN with Blueberry extract dose1500mg/kg BW; Red arrow shows the expression of IL-1β marked by brown color on cells

Table 1 The mean percentage of IL-1β expression in rat's kidney

Groups	Expression of IL-1β (% area) Mean ± SD	Percentage Decreases against Positive Control (%)
A (Negative control)	0.52 ± 0.11^{a}	-
B (AGN Positive control)	$12.12 \pm 0,37^{e}$	-
C (500 mg/kg)	8.18 ± 0.51^{d}	32.50
D(1000 mg/kg)	6.00 ± 0.16^{c}	50.49
E(1500 mg/kg)	3.42 ± 0.26^{b}	71.78

The Values given are the average of four replicates; mean ± SD value followed by the different letters in the same vertical column are significantly different ($p < 0.05$)

group "A" (Figure 1 A-E). Further, significant inhibition of AGN occurrence was demonstrated in the group treated with the blueberry extract (Table 1). Streptokinase converts plasminogen into plasmin, activating the complement reaction (C3 and C5a), increasing renal capillary permeability, and triggering an increase in IL-1β level. Damaged tissue during inflammation also causes the activation of macrophages and the formation of ROS that triggers the release of proinflammatory cytokines, one of which is IL-1β (Soderholm et al. 2018). Proinflammatory cytokine production can continuously lead to a prolonged inflammatory phase and recovery time.

The IL-1β expression in group A showed the lowest percentage in all groups, while group B showed the highest percentage. The expression of IL-1β significantly decreased in the blueberry therapy groups as compared to the positive (B) control group ($p<0.05$). Among blueberry groups, group E have significantly lower IL-1β expression compared with group C and D ($p<0.05$); however, the percentage of IL-1β expression was still significantly higher compared with the negative control group (A) ($p<0.05$).

Decreased IL-1β levels in the blueberry therapy groups were proportional to the increasing dose of blueberry extract. Blueberry extract therapy doses of 500 mg/kg, 1000 mg/kg, and 1500 mg/kg were able to decrease the IL-1β expression by 32.50%, 50.49%, and 71.78%, respectively as compared to the positive control. Findings of the study indicated that blueberry extract has a positive effect on preventing inflammatory response of AGN. The blueberry anthocyanin content has been reported as 487 mg/100g, which is the highest among berry fruits (Kalt et al. 2020). Antioxidant and anti-inflammatory effects of berry fruits are related to the concentration of anthocyanins. The role of blueberry in the treatment of cardiovascular (Zhu et al. 2013) and kidney disease (Elks et al. 2011) was also well established. Berry fruit acts as an anti-inflammatory compound by inhibiting the conversion of arachidonic acid into prostaglandin (PG) E2 through the synthesis of cyclooxygenase (COX) enzymes and also by suppressing the formation of antigen-antibody complexes occurring in the glomerulus (Denis et al. 2015). Thus, it can suppress complement activation and release of IL-1β cytokines.

3.2 Effect of Blueberry extract against Inflammatory Cells

The morphological evaluation of the rat's kidney showed abnormal structure due to streptokinase induction (Figure 2 B-E). The microscopic analysis of the kidney showed inflammatory cell infiltration, tubular epithelial erosion, interstitial hemorrhage, and glomerular hypertrophy. Nevertheless, compared to streptokinase-administered rats, the blueberry post-treated animals exhibited improvement in histological changes (Figure 2 C-E). Further, in the blueberry therapy groups, Bowman's space becomes wider, impairment in epithelial tubular and glomerular cells, and the presence of few inflammatory cells have been also reported. Glomerulonephritis was characterized by the increasing cell inflammation and the addition of endothelial and epithelial cells in the glomerulus that cause glomerular enlargement and narrowing of Bowman's space (Pirozzi et al. 2018).

The pathogenic mechanism of streptokinase-induced acute glomerulonephritis is started with an accumulation of streptokinase antigen in the glomeruli leading to complement activation, deposition of C3 and IgG, mobilization of immune cells, and following aggravation of glomerular inflammation (Nordstrand et al. 2009). Leukocytes, as one of the immune cells, patrol normal glomerulus. Meanwhile, neutrophil cells become the first responders against inflammation in the glomerular. Furthermore, other immune cells i.e. CD4+ and CD8+ T-cells, macrophages, and dendritic cells, alleviate local inflammation and systemic immunity (Richard Kitching and Hutton, 2016).

A quantitative assessment of inflammatory cell density was achieved by calculating the number of inflammatory cell occurrences on the kidney micrograph. The highest total of inflammatory cells was in group B, and these are significantly different from the negative control group (A) and blueberry therapy group (Table 2 C-E). The injection of streptokinase triggers the release of proinflammatory cytokines as inflammatory cell chemotactic which resulted in infiltration of inflammatory cells, i.e., migration from monocyte and macrophages to the place of complement activation. Damaged tissue during inflammation also causes the activation of macrophages (Soderholm et al. 2018).

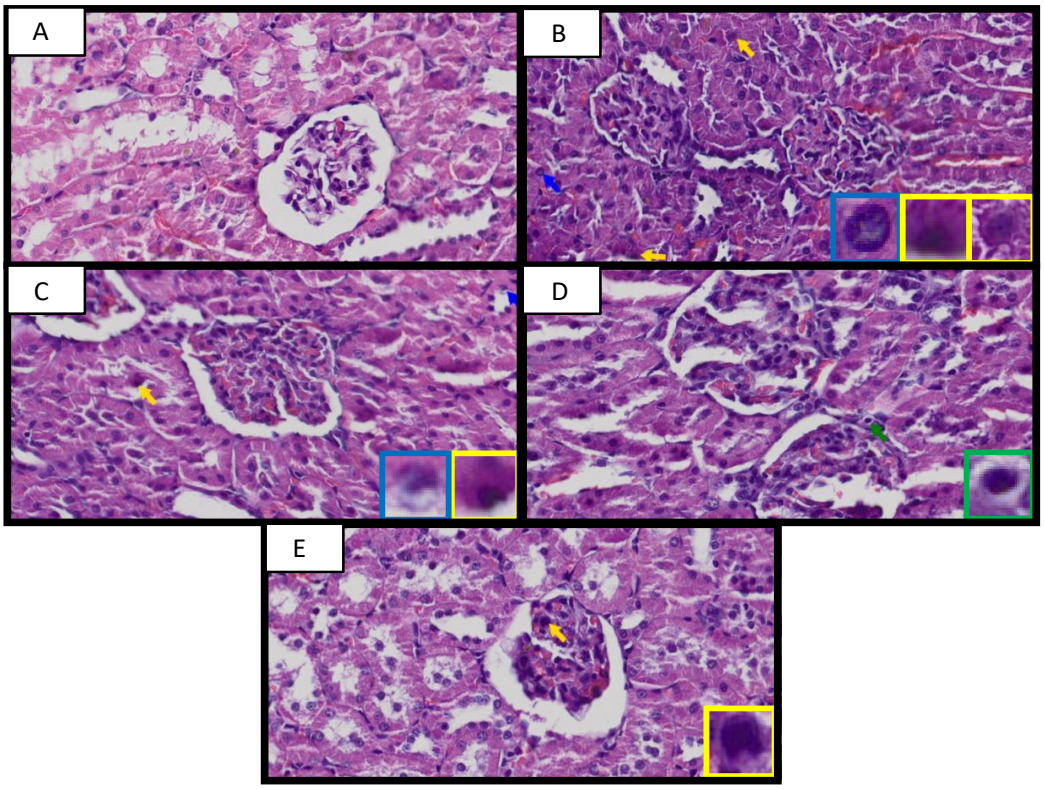

Figure 2 Blueberry therapy impairment renal tissue damaged due to streptokinase-induced glomerulonephritis model in the rat (H&E staining: magnification of 400x); here Yellow arrow and yellow box show the macrophage; Blue arrow and blue box show the neutrophil; Green arrow and green box shows the lymphocyte, Negative control group (A); Positive control group (B); Blueberry extract dose 500mg/kg (C); Blueberry extract dose 1000mg/kg (D); Blueberry extract dose 1500mg/kg (E)

Table 2 Mean of total inflammatory cell count on rat's kidney histopathology

Group	Total of Inflammatory Cell (cell/mm^2)	Percentage Decreases against Positive Control (%)
A (Negative control)	2.25 ± 0.50^a	-
B (Positive control)	7.25 ± 2.06^b	-
C (BB 500 mg/kg)	4.25 ± 0.95^a	41.38
D (BB 1000 mg/kg)	3.50 ± 1.29^a	51.72
E (BB 1500 mg/kg)	3.25 ± 0.95^a	55.17

The Values given are the average of four replicates; mean ± SD values followed by the different letters in the same vertical column are significantly different ($p < 0.05$)

The total of inflammatory cells in the blueberry therapy group gradually declined following the increased dose of the blueberry extract but this difference is not statistically significant ($p>0.05$). Blueberries are rich in anthocyanidins and anthocyanins content and the anti-inflammatory properties of blueberries might be due to the presence of these chemicals (Pervin et al. 2016; Khoo et al. 2017; Fauzi et al. 2021). The result obtained in this study suggests that blueberry extract has potential anti-inflammatory activities against AGN, and abolishes inflammatory cell infiltration. According to Subarnas and Wagner (2000), a higher concentration of anthocyanins had anti-inflammatory activity by restraining cyclooxygenases and cytokines' pro-inflammation production. Further, anthocyanin inhibited the change of arachidonic acid into prostaglandin (PG) E2 through a synthesis of the cyclooxygenase (COX) enzyme. It suppressed the formation of antigen-antibody complexes that occur in the glomerulus (Matout et al. 2019). It complements the activation and release of cytokines. Inhibition of the release of cytokines causes infiltration of inflammatory cells so that the reduced inflammatory response can make cells functional again. The role of other phenolic content cannot be denied because they might also involve in the anti-inflammatory mechanisms.

Conclusion

Results of the study can be concluded that the extract of Blueberry has a significant anti-inflammatory effect on acute glomerulonephritis (AGN) animal model with an optimum dose was 1500 mg/kg. Furthermore, the results of this study encourage the use of blueberry extract as alternative supplementation therapy for coping with acute glomerulonephritis (AGN). Future analysis of the bioactive *V. corymbosum* is essential to prove its mechanism in kidney injury.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Authors Contribution

AF, FSP: designed the conceptualization, investigation, and manuscript writing; AN, AFP: conducted investigation, data curation, and writing draft preparation. NT: Validating, editing, and reviewing. The manuscript's final version was approved by all the authors.

References

Ahmet, I., Spangler, E., Shukitt-Hale, B., Juhaszova, M., et al. (2009). Blueberry-enriched diet protects rat heart from ischemic damage. *PloS One*, *4*(6). https://doi.org/10.1371/JOURNAL.PONE.0005954

Akdis, M., Aab, A., Altunbulakli, C., Azkur, K., et al. (2016). Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases. *Journal of Allergy and Clinical Immunology*, *138*(4), 984–1010. https://doi.org/10.1016/J.JACI.2016.06.033

Alkhalf, M. I., & Khalifa, F. K. (2018). Blueberry extract attenuates γ-radiation-induced hepatocyte damage by modulating oxidative stress and suppressing NF-κB in male rats. *Saudi Journal of Biological Sciences*, *25*(7), 1272–1277. https://doi.org/10.1016/J.SJBS.2018.07.002

Alzahrani, Y., Colorado, L. H., Pritchard, N., & Efron, N. (2021). Longitudinal changes in Langerhans cell density of the cornea and conjunctiva in contact lens-induced dry eye. *Clinical & experimental optometry*, *100*(1), 33–40. https://doi.org/10.1111/cxo.12399.

Brodsky, S. V., & Nadasdy, T. (2017). Acute Poststreptococcal Glomerulonephritis. *Bacterial Infections and the Kidney*, 1–36. https://doi.org/10.1007/978-3-319-52792-5_1

Brown, S. (2016). Glomerular Disease in Small Animals - Urinary System - MSD Veterinary Manual. In *MSD Manual Veterinary Manual*. College of Veterinary Medicine, University of Georgia. Retrived from https://www.msdvetmanual.com/urinary-system/noninfectious-diseases-of-the-urinary-system-in-small-animals/glomerular-disease-in-small-animals

Denis, M. C., Desjardins, Y., Furtos, A., Marcil, V., et al. (2015). Prevention of oxidative stress, inflammation and mitochondrial dysfunction in the intestine by different cranberry phenolic fractions. *Clinical Science*, *128*(3), 197–212. https://doi.org/10.1042/CS20140210

Elks, C. M., Reed, S. D., Mariappan, N., Shukitt-Hale, B., Joseph, J. A., Ingram, D. K., & Francis, J. (2011). A Blueberry-Enriched Diet Attenuates Nephropathy in a Rat Model of Hypertension via Reduction in Oxidative Stress. *PLOS ONE*, *6*(9), e24028. https://doi.org/10.1371/JOURNAL.PONE.0024028

Fauzi, A., Vidiastuti, D., Noviatri, A., & Rizal, M. Y. (2021). Blueberry Extract (*Vaccinium corymbosum*) Attenuates Tnf-α Expression and Renal Inflammatory Cell Counts in Rats Models of Acute Kidney Injury. *Advances in Animal and Veterinary Sciences*, *9*(7), 1087–1094. https://doi.org/10.17582/JOURNAL.AAVS/2021/9.7.1087.1094

Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., & Zamora-Ros, R. (2020). Recent Research on the Health Benefits of Blueberries and Their Anthocyanins. *Advances in Nutrition*, *11*(2), 224. https://doi.org/10.1093/ADVANCES/NMZ065

Keragala, C. B., Draxler, D. F., McQuilten, Z. K., & Medcalf, R. L. (2018). Haemostasis and innate immunity – a complementary relationship. *British Journal of Haematology*, *180*(6), 782–798. https://doi.org/10.1111/BJH.15062

Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research*, *61*(1), 1361779. https://doi.org/10.1080/16546628.2017.1361779

Matout, M., Halme, A. S., & Wiseman, J. (2019). A case of acute kidney injury secondary to black cherry concentrate in a patient with chronic kidney disease secondary to type 2 diabetes mellitus. *CEN Case Reports*, *8*(3), 212–215. https://doi.org/10.1007/S13730-019-00396-2

Medica, D., Franzin, R., Stasi, A., Castellano, G., et al. (2021). Extracellular Vesicles Derived from Endothelial Progenitor Cells Protect Human Glomerular Endothelial Cells and Podocytes from Complement- and Cytokine-Mediated Injury. *Cells*, *10*(7). https://doi.org/10.3390/CELLS10071675

Michalska, A., & Łysiak, G. (2015). Bioactive Compounds of Blueberries: Post-Harvest Factors Influencing the Nutritional Value of Products. *International Journal of Molecular Sciences*, *16*(8), 18642–18663. https://doi.org/10.3390/IJMS160818642

Nair, A. R., Elks, C. M., Vila, J., Del Piero, F., Paulsen, D. B., & Francis, J. (2014). A blueberry-enriched diet improves renal function and reduces oxidative stress in metabolic syndrome animals: potential mechanism of TLR4-MAPK signaling pathway. *PloS One*, *9*(11). https://doi.org/10.1371/JOURNAL.PONE.0111976

Nordstrand, A., Norgren, M., & Holm, S. E. (2009). Pathogenic Mechanism of Acute Post-Streptococcal Glomerulonephritis. *Scandinavian Journal of Infectious Diseases*, *31*(6), 523–537. https://doi.org/10.1080/00365549950164382

Noris, M., & Remuzzi, G. (2013). Overview of Complement Activation and Regulation. *Seminars in Nephrology*, *33*(6), 479–492. https://doi.org/10.1016/J.SEMNEPHROL.2013.08.001

Pervin, M., Hasnat, M. A., Lim, J. H., Lee, Y. M., Kim, E. O., Um, B. H., & Lim, B. O. (2016). Preventive and therapeutic effects of blueberry (*Vaccinium corymbosum*) extract against DSS-induced ulcerative colitis by regulation of antioxidant and inflammatory mediators. *The Journal of Nutritional Biochemistry*, *28*, 103–113. https://doi.org/10.1016/J.JNUTBIO.2015.10.006

Pirozzi, N., Stoppacciaro, A., & Menè, P. (2018). Dominant C3 glomerulopathy: new roles for an old actor in renal pathology. *Journal of Nephrology*, *31*(4), 503–510. https://doi.org/10.1007/S40620-017-0458-Y/TABLES/1

Richard Kitching, A., & Hutton, H. L. (2016). The players: Cells involved in glomerular disease. *Clinical Journal of the American Society of Nephrology*, *11*(9), 1664–1674. https://doi.org/10.2215/CJN.13791215

Shilpi, B., Sourav, B., & Arijit, D. (2013). Isolation, Screening and Characterization of β-Haemolytic Streptococci with Potential of Streptokinase Production. *International Research Journal of Biological Sciences*, *2*(4), 63–66.

Shomer, N. H., Worthington, K. H. A., Hickman, D. L., Jonnalagadda, M., et al. (2020). Review of rodent euthanasia methods. *Journal of the American Association for Laboratory Animal Science*, *59*(3), 242–253. https://doi.org/10.30802/AALAS-JAALAS-19-000084

Soderholm, A. T., Barnett, T. C., Sweet, M. J., & Walker, M. J. (2018). Group A streptococcal pharyngitis: Immune responses involved in bacterial clearance and GAS-associated immunopathologies. *Journal of Leukocyte Biology*, *103*(2), 193–213. https://doi.org/10.1189/JLB.4MR0617-227RR

Subarnas, A., & Wagner, H. (2000). Analgesic and anti-inflammatory activity of the proanthocyanidin shellegueain A from Polypodium feei METT. *Phytomedicine*, *7*(5), 401–405. https://doi.org/10.1016/S0944-7113(00)80061-6

VanDeVoorde, R. G. (2015). Acute Poststreptococcal Glomerulonephritis: The Most Common Acute Glomerulonephritis. *Pediatrics in Review*, *36*(1), 3–13. https://doi.org/10.1542/PIR.36.1.3

Zhu, Y., Ling, W., Guo, H., Song, F., et al. (2013). Anti-inflammatory effect of purified dietary anthocyanin in adults with hypercholesterolemia: a randomized controlled trial. *Nutrition, Metabolism, and Cardiovascular Diseases* , *23*(9), 843–849. https://doi.org/10.1016/J.NUMECD.2012.06.005