Effectiveness of Anthocyanins on Kidney Fibrosis; A Systemic Literature Review

Evi Lusiana[1], Aida Nur Azizah[2], Arya Putera Islami[2]

[1]Bagian Farmakologi, Fakultas Kedokteran, Universitas Sriwijaya, Palembang, Indonesia
[2]Pendidikan Dokter, Fakultas Kedokteran, Universitas Sriwijaya, Palembang, Indonesia

ABSTRACT

Renal fibrosis refers to the abnormal accumulation of extracellular matrix during the process of tissue repair. Kidney fibrosis is mostly caused by chronic inflammation, which leads to tissue remodeling and subsequently impairs kidney function, resulting in a reduction in the glomerular filtration rate (GFR). Reduced glomerular filtration rate (GFR) can elevate blood concentrations of urea and creatinine. The chemicals known as anthocyanins possess anti-inflammatory and antioxidant characteristics, hence potentially enhancing renal function. This study was conducted based on a literature review approach, utilizing journal papers published over the past decade (2015-2024) obtained from Pubmed, Science Direct, and Google Scholar. This article aims to represent the diverse scientific investigations made on the efficacy of anthocyanins in enhancing renal function in cases with renal fibrosis. The present study indicates that anthocyanins have a positive impact on kidney function by effectively lowering urea and creatinine levels in individuals with renal fibrosis.
Keywords: Anthocyanin, Ureum, Creatinine, Renal Fibrosis

Correspondence: mrs.evilusiana@fk.unsri.ac.id

Introduction

The kidneys are organs that regulate acid-base balance, water and electrolyte balance, hormone release, and excretion of metabolic waste products.[1] When the kidneys undergo inflammation, infection, or obstruction, there will be an impairment in kidney function. Kidney fibrosis refers to the morphological changes that occur in the kidneys, which represent the ultimate stage of kidney failure.[2]

Kidney fibrosis is the accumulation of extracellular matrix as a pathological form of tissue repair process. The deposition of extracellular matrix occurs as a result of tissue remodeling and activation of myofibroblasts, which is triggered by chronic inflammation, [3] thus disrupting kidney function. Disruption of kidney function leads to a reduction in the glomerular filtration rate (GFR). This can result in elevated amounts of urea and creatinine in the bloodstream. [4–7]

Anthocyanins are a class of chemical substances that are formed from flavonoids. [8] Anthocyanins are pigments found in plants including purple sweet potatoes, purple cabbage, blackcurrants, and other plants that are purple or blue in color.[9] Anthocyanins have the ability to enhance kidney function due to their anti-inflammatory and antioxidant properties. [10] The specific anti-inflammatory features in question are to the ability of anthocyanins to suppress the synthesis of pro-inflammatory cytokines.[11] Anthocyanins possess antioxidant properties since they have the ability to decrease the quantity of active free radicals by providing electrons to reactive oxygen species (ROS). [12,13]

Anthocyanins have a considerable effect in reducing proteinuria, [14] serum creatinine, [15] and transforming growth factor beta 1 (TGFβ1).[16] However, additional investigation is required to evaluate the efficacy of anthocyanins in alleviating kidney fibrosis. This article aims to clarify the scientific research regarding to the efficacy of anthocyanins in enhancing kidney function in cases with kidney fibrosis.

Method

This study conducted a systematic literature review method using the PRISMA flow chart with data sources in the form of journal articles in the last 10 years, from 2015 to 2024, which were accessed through PubMed, ScienceDirect, and Google Scholar. Several keywords in Indonesian are 'antosianin, ureum, kreatinin, fibrosis ginjal,' and in English, 'anthocyanin, urea, creatinine, kidney fibrosis, anthocyanin, serum urea, serum creatinine, and kidney fibrosis'

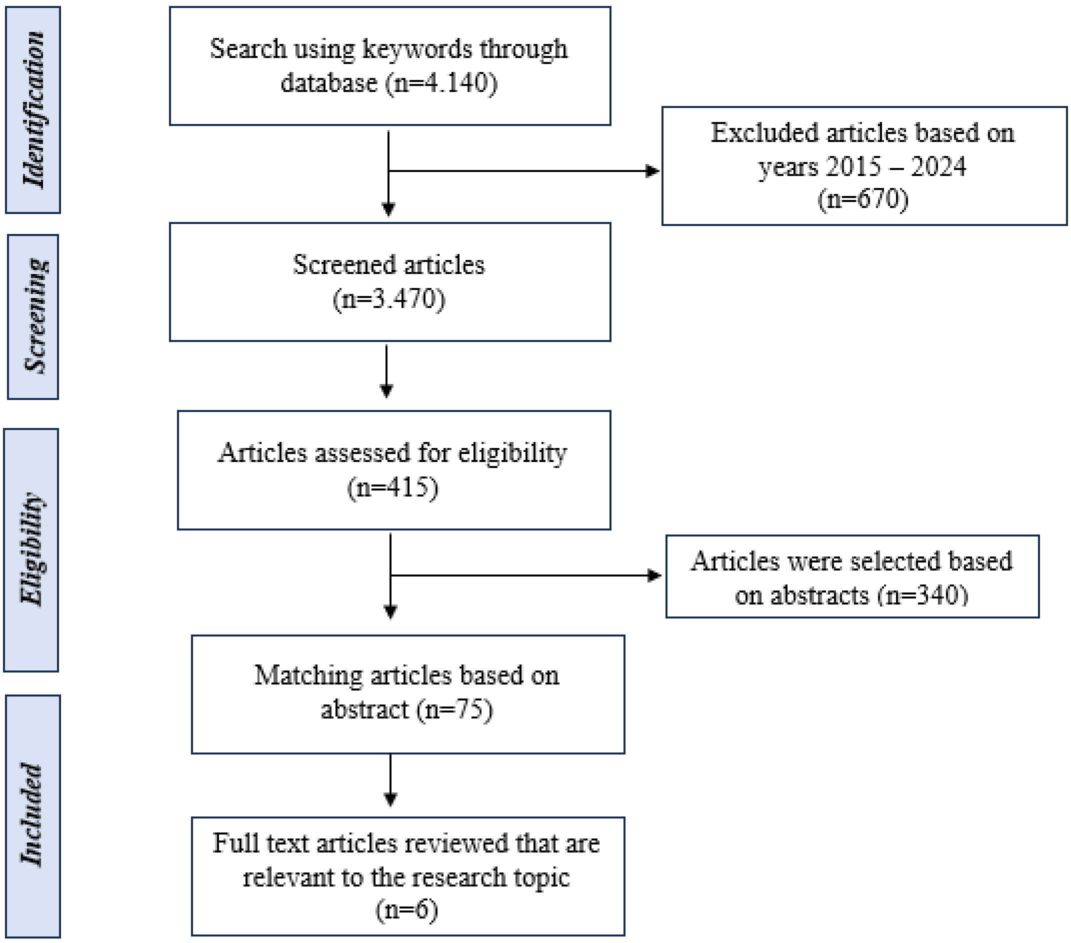

Figure 1. PRISMA *flow chart*

Result and Discussion

This study employs a systematic literature review approach, specifically focusing on studies that utilize in vivo experimental methods. The article employs experimental animals in the form of rats and mice as research subjects. These animals are frequently utilized as experimental subjects. [17] Rat and Mice, commonly used as experimental animals, are frequently employed to develop disease models, including the experimental animal model of fibrosis.

There are several types of fibrosis induction methods used in the articles. The first induction method is carbon tetrachloride (CCl_4) induction, especially for liver fibrosis. CCl_4 can cause liver fibrosis because the compound is a carcinogenic agent.[18] In addition, there are also articles that use the ischemia-reperfusion (IR) induction type. This type of induction is a common technique used to induce experimental animals into acute renal failure (ARF) and kidney fibrosis models.[19] Another type of induction used is using ethanol. Ethanol can cause renal fibrosis through the activation of Nox2/4-mediated DNA methylation of SMAD7. [20] In addition, renal fibrosis is also formed by inducing hyperuricemia in experimental animals, because this condition can progress to renal fibrosis.[21] Hyperuricemia can be induced in experimental animals using a high yeast diet and potassium oxonate solution. A high yeast diet can cause colonization of Saccharomyxes cerevisiae bacteria which can increase uric acid secretion in experimental animals.[22] Potassium oxonate solution acts as a selective competitive inhibitor of the uricase enzyme, thereby increasing uric acid levels in the blood (hyperuricemia).[23] Another induction method used is by utilizing ochratoxin-A. This compound is nephrotoxic because it can induce epithelial-to-mesenchymal transition which can form renal fibrosis. [24]

Table 1. Research design in each article

Num	Author & Year	Subject	Induction method	Type of intervention	Dosage & Duration of Treatment	Assessed parameters
1	Li, *et all* (2020)	Male mice strain C57BL/6 (n = 26), Age = 10-12 weeks	*Ischemia-reperfusion* (IR)	Mice were given cyanidin-3-arabinoside, cyanidin-3-glucodise, cyaniding-3-galactoside, and anthocyanin (a mixture of three cyanidins) orally.	50mg/g BW anthocyanin followed by 25mL/g BW saline 2x/day for 14 days after IR induction	• Serum creatinine and BUN levels • IL-1β, IL-6, TNF-α, MCP-1 levels • GSH, SOD, CAT levels • MDA, TBARS levels • Renal histopathology
2	Alnamshan (2022)	Male rat (n = 40), Age = 170 ± 20 gram	Rat were given ethyl alcohol orally at a dose of 6 mg/kgBW/day for 5 weeks.	Oral administration of black rice ethanol extract	100 mg/kgBW/day and 200 mg/kgBW/day for 5 weeks	• Urea, creatinine, uric acid, protein, albumin, albumin/ globulin ratio • MDA, GSH, SOD levels • Histopathological picture of the kidneys
3	Popović, *et all* (2018)	Male Wistar rats (n = 32), Age = 160 days, BW = 240 ± 20 grams	Rat were given CCl4 intraperitoneally at a dose of 3 mL/kgBW. CCl4 was mixed with olive oil at a ratio of 1:1.	Rat were given concentrated bilberry extract orally.	200mg/kgBW/day for 7 days	• Creatinine, urea, uric acid, β2-microglobulin, lipocalin-2, TIM1/KIM1 levels • CAT, SOD, POD, GPx, GST, GR, GSH, GSSG levels • TNF-α, NO2-, MPO levels • Histopathology, immunohistochemistry, and morphometric features
4.	Qian, *et all* (2019)	ICR male mice (n=40),	Mice were fed a high yeast diet with a chow diet.	Mice were given low and high doses of anthocyanin	400 mg/kgBW/day and 800 mg/kgBW/day for 3 weeks	• Serum levels of uric acid, urea, and creatinine • SOD expression and activity

Num	Author & Year	Subject	Induction method	Type of intervention	Dosage & Duration of Treatment	Assessed parameters
		BW = 28 ± 2 grams		orally for 3 weeks.		• Kidney and liver histopathology • Caspase-1, IL-β, and TNF-α expression • IL6, IL8, IL18 mRNA expression
5.	Zhang, *et all* (2019)	Kunming strain male mice (n=48), Age = 8 weeks	Mice in all groups, except the normal control group, were given potassium oxonate solution at a dose of 250 mg/kgBW/day orally, once a day for 7 days.	Mice were given highly acylated anthocyanins (HAA-PSP) orally.	HAA-PSP = 25 mg/kgBW Single dose	• Serum uric acid levels • Serum urea and creatinine levels • T-SOD and MDA activities • Renal histopathology • Expression of NF-κB, TNF-α, TGF-β1, IL-6, and IL-1β
6.	Damiano, *et all* (2019)	Male Sprague-Dawley rats (n=24), Age = 10 weeks BW = 240 ± 20 grams	Rat were given ochratoxin A (OTA) at a dose of 0.5 mg/kgBW orally for 14 days.	Mice were given red orange and lemon extract (RLE) orally for 14 days.	RLE = 90 mg/kgBW for 14 days	• Rat body weight • Serum urea and creatinine levels • GFR • SOD, CAT, GSH enzyme activities • MDA levels • Histopathological features

All experimental animals were given oral anthocyanin supplementation with various doses. The anthocyanins used came from various sources, such as Aronia Melanocarpa berries, black rice, bilberry extract, blackcurrant, purple sweet potato, and red orange and lemon extracts.[25–30]

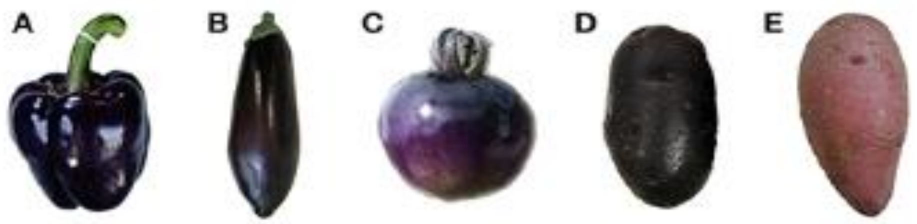

Figure 2. Examples of plants rich in anthocyanins
(A) purple pepper, (B) purple eggplant, (C) purple tomato, (D) purple sweet potato, (E) red sweet potato.(source: Liu Y, 2018)[31]

Anthocyanins, being water-soluble pigments, are responsible for the red, purple, and blue colours found in several flowers and fruits. [32] Anthocyanin synthesis is also stimulated during periods of stress or infection by pathogens, and it functions as a defense mechanism for plants against oxidative stress, such as excessive radiation, when their ability to fix carbon is diminished. [33] Anthocyanins provide protection to photosynthetic tissues by absorbing light, which in turn reduces light stress. Additionally, they serve as antioxidants.[34]

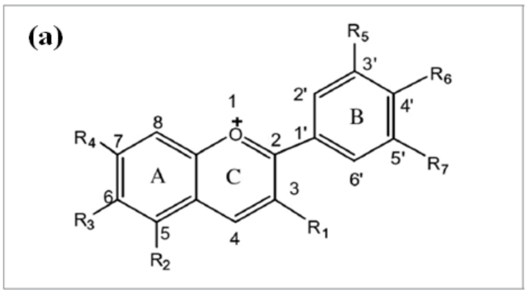

Figure 3. Chemical structure of Anthocyanin

Chemical structure of anthocyanins; Anthocyanin molecules occur naturally in plants as glycosides (glyc) in which the anthocyanidin is bound to a sugar moiety, with glucose, galactose, rhamnose, xylose, or arabinose bound to the aglycone. Chemically, anthocyanidins are polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrylium: 2 benzoyl rings (A and B) separated by a heterocyclic ring (C).[35]

Anthocyanins, in addition to their physiological function in plants, have been associated with a reduced risk of cancer, heart disease, and chronic diseases, including kidney fibrosis.[36] Anthocyanins primarily function as antioxidants and anti-inflammatory agents. The kidneys are the primary target organ for anthocyanins. This is demonstrated by the fact that the concentration of anthocyanins in the kidneys of mice is two to four times higher than in the liver of mice following anthocyanin administration. This indicates that the kidneys have a more effective capacity to uptake and absorb anthocyanins in the short term compared to the liver.

The metabolism of anthocyanin in renal tubular cells is mediated by the enzyme catechol-O-methyl transferase (COMT). Anthocyanin excretion is rapid, taking approximately 20 minutes in its intact form or after being metabolized from bile into urine. [37] Administering anthocyanin can effectively suppress the development of ischemia and necrotic processes in kidney fibrosis. [15] This system operates by mitigating oxidative stress in the kidneys, thereby halting the fibrosis process and restoring kidney tissue. However, anthocyanins have significant inhibitory effects on the production of connective tissue growth factors in fibrosis, which is sometimes referred to as cellular communication network factor 2 (CCN2). [38] Each paper assesses multiple parameters, including pre-and post-intervention serum urea and creatinine levels. Additional characteristics that are assessed include levels of proinflammatory cytokines and kidney histology.

Table 2. Data from Research Evaluation Results

Num	Author & Year	Type of processing	Active compounds	Evaluation result		P Value*
				Urea Level	Creatinine level	
1.	Li, *et all* (2020)	Berry anthocyanin *Aronia Melanocarpa*	Anthocyanins (cyanidin-3-arabinoside, cyanidin-3-glucodise, dan cyaniding-3-galactoside)	There was a significant decrease in urea levels after administration of anthocyanins.	There was a significant decrease in serum creatinine levels after anthocyanin administration.	p<0,05 (significant)
2.	Alnamshan (2022)	Black rice extract	Flavonoids Anthocyanins (cyanidin chloride)	There was a significant decrease in urea levels after administration of black rice ethanol extract.	There was a significant decrease in serum creatinine levels after administration of black rice ethanol extract.	p<0,05 (significant)
3.	Popović, *et all* (2018)	Bilberry extract	Anthocyanins	There was a significant decrease in urea levels after administration of bilberry extract.	There was a significant decrease in serum creatinine levels after administration of bilberry extract.	p<0,05 (significant)
4.	Qian, *et all* (2015)	Anthocyanin powder derived from bilberry and blackcurrant	Anthocyanins	There was a significant decrease in urea levels after administration of anthocyanins.	There was a significant decrease in serum creatinine levels after anthocyanin administration.	p<0,05 (significant)
5.	Zhang, *et all* (2019)	*High acylated anthocyanins-purple sweet potato* (HAA-PSP)	Anthocyanins	There was a significant decrease in urea levels after administration of HAA-PSP.	There was a significant decrease in serum creatinine levels after administration of HAA-PSP.	p<0,05 (significant)
6.	Damiano, *et all* (2019)	Ekstrak jeruk merah dan lemon (RLE)	Cyanidine 3-glucoside (C3G)	There was a significant decrease in urea levels after administration of RLE.	There was a significant decrease in serum creatinine levels after administration of RLE.	p<0,05 (significant)

**one-way ANOVA followed by post hoc*

According to the findings from the article evaluation presented in Table 2, it can be inferred that all research outcomes indicate that anthocyanins have the potential to enhance kidney function by reducing levels of serum urea and creatinine following intervention by anthocyanins. The statistical analysis of differences between groups was assessed using one-way ANOVA, followed by a post hoc test. The results were judged significant with p-value of less than 0.05.

Li et al. (2020) found that anthocyanins have the potential to eliminate pathological damage such as tubular cell swelling, atrophy, dilatation, and glomerular necrosis in the kidneys caused by IR induction.[25] Anthocyanins possess potent anti-inflammatory effects. Anthocyanins have the ability protect the kidneys against additional harm and decrease the accumulation of urea and creatinine in the bloodstream. Anthocyanins are recognized as potent antioxidants. Oxidative damage is the main contributor to the development of kidney fibrosis. Anthocyanins have the ability to reduce oxidative stress, thereby protecting kidney cells towards injury and decelerating the progression of fibrosis. Consequently, this can lead to a decrease in levels of urea and creatinine.

Anthocyanins has anti-fibrotic properties which may inhibit the development of scar tissue in kidney fibrosis. Anthocyanins have the ability to decrease fibrosis, which in turn can decelerate the deterioration of renal function and therefore lower an increase in urea and creatinine levels. Anthocyanins can also enhance the endothelial function of blood arteries, hence influencing the blood flow to the kidneys.

Conclusion and Suggestion

A conclusion can be inferred that anthocyanins have the potential to enhance kidney function in rat and mice models of kidney fibrosis. Studies on animal models of kidney fibrosis have shown that anthocyanin treatment can yield positive outcomes by reducing serum creatinine levels, which are important indicators of kidney failure. Possible mechanisms include the anti-inflammatory, antioxidant, and anti-fibrotic characteristics of anthocyanins. These features have the ability to protect kidney cells against oxidative damage, diminish inflammation, and impede the progression of kidney fibrosis. A preclinical trial could involve testing anthocyanin supplementation in an animal model of renal fibrosis. This would assist in assessing the potential of anthocyanins to ameliorate renal fibrosis prior to clinical trials.

References

1. Rosida A, Pratiwi ewi IN. Pemeriksaan Laboratorium Sistem Uropoetik PK UNLAM. 2019. 60 p.
2. Liu BC, Lan HY, Lv LL. Renal Fibrosis: Mechanisms and Therapies. Springer; 2019.
3. Panizo S, Martínez-Arias L, Alonso-Montes C, Cannata P, Martín-Carro B, Fernández-Martín JL, et al. Fibrosis in chronic kidney disease: Pathogenesis and consequences. Int J Mol Sci. 2021;22(1):1–19.
4. Huang R, Fu P, Ma L. Kidney fibrosis: from mechanisms to therapeutic medicines. Signal Transduct Target Ther. 2023;8(1).
5. Kaufman DP, Basit H, Knohl SJ. Physiology, glomerular filtration rate. 2018;
6. Gounden V, Bhatt H, Jialal I. Renal Function Tests. University of KwaZulu Natal: StatPearls Publishing, Treasure Island (FL); 2022.
7. Cho MH. Renal fibrosis. Korean J Pediatr. 2010;53(7):735.
8. Mattioli R, Francioso A, Mosca L, Silva P. Anthocyanins: A

Comprehensive Review of Their Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative Diseases. Molecules. 2020;25(17).

9. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017;61(1).
10. Ma Z, Du B, Li J, Yang Y, Zhu F. An insight into anti-inflammatory activities and inflammation related diseases of anthocyanins: A review of both in vivo and in vitro investigations. Int J Mol Sci. 2021;22(20).
11. Li S, Wu B, Fu W, Reddivari L. The anti-inflammatory effects of dietary anthocyanins against ulcerative colitis. Int J Mol Sci. 2019;20(10):2588.
12. Ifadah RA, Rizkia P, Wiratara W, Anam C. Ulasan Ilmiah : Antosianin dan Manfaatnya untuk Kesehatan. 2021;3(2):11–21.
13. Qin Y, Zhai Q, Li Y, Cao M, Xu Y, Zhao K, et al. Cyanidin-3-O-glucoside ameliorates diabetic nephropathy through regulation of glutathione pool. Biomedicine and Pharmacotherapy. 2018;103(88):1223–30.
14. Qi SS, He J, Yuan LP, Le Wu J, Zu YX, Zheng HX. Cyanidin-3-glucoside from black rice prevents renal dysfunction and renal fibrosis in streptozotocin-diabetic rats. J Funct Foods. 2020;72:104062.
15. Herawati ERN, Santosa U, Sentana S, Ariani D. Protective effects of anthocyanin extract from purple sweet potato (Ipomoea batatas L.) on blood MDA levels, liver and renal activity, and blood pressure of hyperglycemic rats. Prev Nutr Food Sci. 2020;25(4):375.
16. Zhang G, Jiang Y, Liu X, Deng Y, Wei B, Shi L. Lingonberry anthocyanins inhibit hepatic stellate cell activation and liver fibrosis via tgfβ/smad/ERK signaling pathway. J Agric Food Chem. 2021;69(45):13546–56.
17. Hickman DL, Johnson J, Vemulapalli TH, Crisler JR, Shepherd R. Commonly used animal models. Principles of animal research for graduate and undergraduate students. 2017;117.
18. Sayed EA, Badr G, Hassan KAH, Waly H, Ozdemir B, Mahmoud MH, et al. Induction of liver fibrosis by CCl4 mediates pathological alterations in the spleen and lymph nodes: The potential therapeutic role of propolis. Saudi J Biol Sci. 2021;28(2):1272–82.
19. Guan Y, Nakano D, Zhang Y, Li L, Tian Y, Nishiyama A. A mouse model of renal fibrosis to overcome the technical variability in ischaemia/reperfusion injury among operators. Sci Rep. 2019;9(1):1–9.
20. Yang Q, Chen HY, Wang J nan, Han HQ, Jiang L, Wu WF, et al. Alcohol promotes renal fibrosis by activating Nox2/4-mediated DNA methylation of Smad7. Clin Sci. 2020 Jan 23;134(2):103–22.
21. Méndez Landa CE. Renal Effects of Hyperuricemia. Contrib Nephrol. 2018;192:8–16.
22. Zhang Y, Chen S, Yuan M, Xu Y, Xu H. Gout and diet: a comprehensive review of mechanisms and management. Nutrients. 2022;14(17):3525.
23. Tang DH, Ye YS, Wang CY, Li ZL, Zheng H, Ma KL. Potassium oxonate induces acute hyperuricemia in the tree shrew (tupaia belangeri chinensis). Exp Anim. 2017;66(3):209–16.
24. Khoi CS, Chen JH, Lin TY, Chiang CK, Hung KY. Ochratoxin A-induced nephrotoxicity: Up-to-date evidence. Int J Mol Sci. 2021;22(20):11237.

25. Li L, Li J, Xu H, Zhu F, Li Z, Lu H, et al. The Protective Effect of Anthocyanins Extracted from Aronia Melanocarpa Berry in Renal Ischemia-Reperfusion Injury in Mice. Mediators Inflamm. 2021;2021.
26. Alnamshan MM. Antioxidant extract of black rice prevents renal dysfunction and renal fibrosis caused by ethanol-induced toxicity. Brazilian Journal of Biology. 2022;82:1–10.
27. Popović D, Kocić G, Katić V, Jović Z, Zarubica A, Veličković LJ, et al. Protective effects of anthocyanins from bilberry extract in rats exposed to nephrotoxic effects of carbon tetrachloride. Chem Biol Interact. 2019;304:61–72.
28. Qian X, Wang X, Luo J, Liu Y, Pang J, Zhang H, et al. Hypouricemic and nephroprotective roles of anthocyanins in hyperuricemic mice. Food Funct. 2019;10(2):867–78.
29. Zhang ZC, Zhou Q, Yang Y, Wang Y, Zhang JL. Highly Acylated Anthocyanins from Purple Sweet Potato (Ipomoea batatas L.) Alleviate Hyperuricemia and Kidney Inflammation in Hyperuricemic Mice: Possible Attenuation Effects on Allopurinol. J Agric Food Chem. 2019;67(22):6202–11.
30. Damiano S, Iovane V, Squillacioti C, Mirabella N, Prisco F, Ariano A, et al. Red orange and lemon extract prevents the renal toxicity induced by ochratoxin A in rats. J Cell Physiol. 2020;235(6):5386–93.
31. Liu Y, Tikunov Y, Schouten RE, Marcelis LFM, Visser RGF, Bovy A. Anthocyanin Biosynthesis and Degradation Mechanisms in Solanaceous Vegetables: A Review. Front Chem. 2018 Mar 9;6.
32. Khoo HE, Azlan A, Tang ST, Lim SM. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food Nutr Res. 2017 Jan 13;61(1):1361779.
33. Fang J. Bioavailability of anthocyanins. Drug Metab Rev. 2014 Nov 1;46(4):508–20.
34. Zhu F, Cai YZ, Yang X, Ke J, Corke H. Anthocyanins, hydroxycinnamic acid derivatives, and antioxidant activity in roots of different chinese purple-fleshed sweetpotato genotypes. J Agric Food Chem. 2010 Jul 14;58(13):7588–96.
35. Pojer E, Mattivi F, Johnson D, Stockley CS. The Case for Anthocyanin Consumption to Promote Human Health: A Review. Compr Rev Food Sci Food Saf. 2013 Sep;12(5):483–508.
36. Laorodphun P, Arjinajarn P, Thongnak L, Promsan S, Swe MT, Thitisut P, et al. Anthocyanin-rich fraction from black rice, Oryza sativa L. var. indica “Luem Pua,” bran extract attenuates kidney injury induced by high-fat diet involving oxidative stress and apoptosis in obese rats. Phytotherapy Research. 2021 Sep 29;35(9):5189–202.
37. Pojer E, Mattivi F, Johnson D, Stockley CS. The Case for Anthocyanin Consumption to Promote Human Health: A Review. Compr Rev Food Sci Food Saf. 2013 Sep;12(5):483–508.
38. Sandoval-Ramírez BA, Catalan U, Fernandez-Castillejo S, Rubio L, Macià A, Solà R. Anthocyanin tissue bioavailability in animals: Possible implications for human health. A systematic review. J Agric Food Chem. 2018;66(44):11531–43.