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Betalains protect various body organs through antioxidant and anti-inflammatory pathways

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ABSTRACT

Betalains are natural coloring pigments with betalamic acid as the core structure of all subclasses. Besides their coloring properties, betalains exhibit various biological activities, including antioxidant and anti-inflammatory properties, which are highly imperative. Further *in-vivo* studies reported that betalains protect various body organs, leading to health enhancement. Body organs, including the heart, liver, kidney, lung, etc., are important for a healthy life. However, these organs can be affected or damaged by various stress factors, toxicants, and harmful substances. Recent studies have claimed that betalains could protect all vital organs of the body through antioxidant and anti-inflammatory mechanisms. This review article described the *in-vivo* antioxidant and anti-inflammatory activities of betalains in various cell-line or animal models. A comprehensive discussion has been provided on the mechanism of action of betalains in protecting various body organs, including cardio-protective effect, hepato-protective ability, renal protection capacity, repro-protective ability, neuro-protective effect, lung protection, and gut protection ability. Finally, future research directions and conclusions have been outlined.

1. Introduction

Betalains are naturally occurring, red-purple, tyrosine-derived pigments belonging to the Caryophyllales family[1]. It is categorized as red-violet betacyanin and yellow-orange betaxanthins[2]. Betalamic acid is at the core of both of these betalain pigments, which are synthesized from tyrosine[3]. The color of beetroot or any other plant in the Caryophyllales family is heavily influenced by the betacyanin/betaxanthin ratio, which typically ranges from 1 to 3[4]. Besides their coloring properties, betalains also possess various biological activities, including antioxidant, anti-inflammatory, anti-obesity, antimicrobial, anti-cancer, anti-diabetic, antihypertensive, etc.[5-8]. These two properties of betalains make them a natural nutraceutical and coloring agent for functional food production and other industrial applications.

Betalains also protect against oxidative damage, inflammation, cellular damage, mitochondrial damage, and chronic diseases that arise[9]. In general, during disease, free radical accumulation is observed over the organs and tissues, which also results in inflammation[10]. The ingestion of betalains modulates numerous signaling pathways, which protect the organs from impairment during pathological conditions. Throughout the antioxidative mechanism, betalains modulate the serum-inducible protein kinase-1 (Nrf2)/antioxidant response elements (ARE) signaling and expression of enzymes

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relevant to organ and disease conditions to enhance Nrf2 transcription and the activity of antioxidative enzymes like glutathione peroxidase (GPx), superoxide dismutases (SOD), and catalase (CAT)[11-12]. In addition to being antioxidants, betalains also regulate inflammation process, such as nuclear factor kappa B (NF-κB) regulation, attenuate pro-inflammatory cytokines, and stimulate anti-inflammatory mediators[13]. It has been reported that the betalains mitigated inflammation and retrieved the function of different organs, including the lung and airways[14], heart[15-16], gut[17], liver[18], kidney[19] and reproductive organs[20]. This suggests that betalains and their derivatives contribute to organ protection in disease conditions via the regulation of antioxidant and anti-inflammatory processes.

In general, most of the reports on betalains' health potential have specifically addressed *in vitro* studies and their relevance as functional ingredients or disease treatment speculation[21]. Yet, they lack insight from *in vivo* studies and the functional efficacy of betalain[12]. Even if some reviews addressed *in vivo* and *ex vivo* studies, a comprehensive articulation of the antioxidant and anti-inflammatory meachnisms and organ protection via these routes remains lacking. Thus, this review aimed to provide a comprehensive and meticulous *in vivo* evidence-based organ-protective effect of betalain and its derivatives, particularly on the heart, liver, kidney, neurons, lung, reproductive, and gut. Atypically, the studies that have chronicled the organ protective properties of betalains, either in cell lines or *in vivo* , are thoroughly evaluated, with a focus on the anti-inflammation and antioxidant regulatory processes as well as the mechanism involved.

2. Betalains chemical composition

Betalains are water-soluble, vacuolar nitrogen-containing pigments made up of betalamic acid as a central structure[1]. Chemically, betalain is composed of two structural groups: betacyanin and betaxanthin[2]. Khan and Giridhar[22] have reviewed the detailed chemistry and biochemistry of plant betalain structure, biosynthesis, and factors influencing betalain production. The biosynthesis of betalain starts with the hydroxylation of tyrosine to form betalamic acid, followed by subsequent cyclization and condensation with imino acids or amino derivatives to form betaxanthin or betacyanin[5]. Further, these two pigments are categorized into several other classes depending on the condensation of the chemical moiety to the core structure. Among them, major betacyanin pigments reported are betanin, isobetanin, and neobetanin, whereas betaxanthins are known to consist of vulgaxanthin I, vulgaxanthin II, and indicaxanthin (Fig. 1)[1,10].

3. Antioxidant activity of betalain

The antioxidant potential of betalain is enhanced when a hydroxyl group is at the C-5 site of the aglycone, while in the case of betaxanthin, it can transfer one electron from the conjugated π-orbitals[23]. In this respect, betaxanthin is usually less potent than betacyanin in terms of antioxidants. Nevertheless, betaxanthins possess more hydroxyl and imino residues that may augment free radical scavenging potential[8]. Several *in vivo* and *ex vivo* studies are focused on the antioxidative effect of betalains and their derivatives (Table 1). According to research on a novel class of dopamine-derived betalains in an animal model, decarboxy-betaxanthins have the most potent antioxidant activity, with down regulation of the expression of the hsp::GFP (green fluorescent protein) in *Caenorhabditis elegan*[24]. Interestingly, it was discovered that both natural and synthetic betalain improve the health of *C. elegan* through antioxidant activity. Additionally, exploration of traditional betalain was evident to promote longevity in nematodes by inhibiting the activation of reactive oxygen species (ROS) and the nuclear factor-E2-related factor 2/Nrf2/serum-inducible protein kinase-1 (SNK-1)

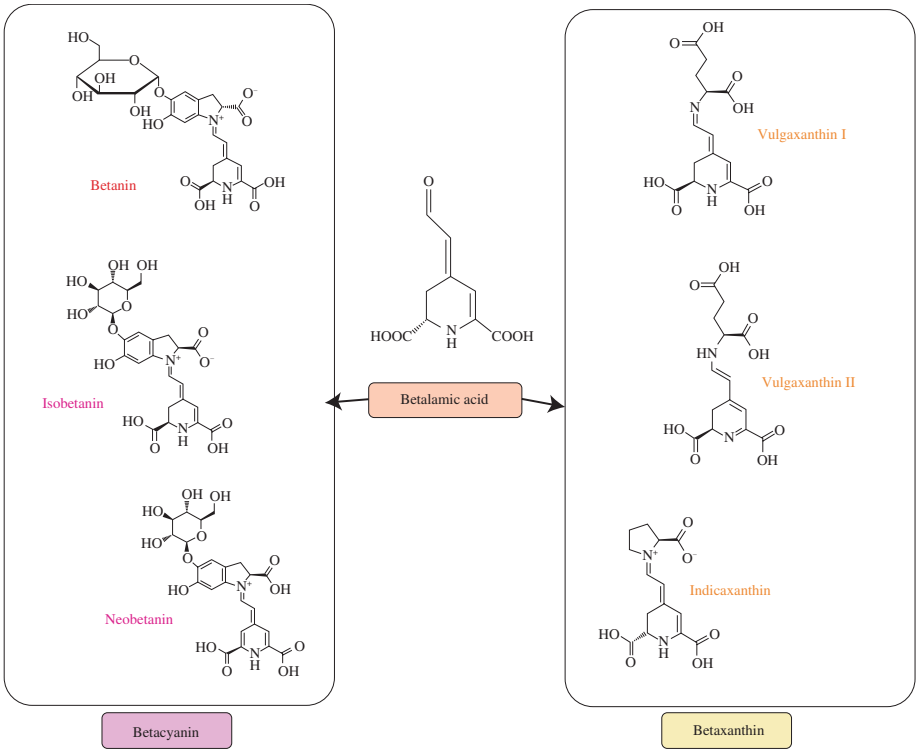

Fig. 1 Betalains and their different classes.

signaling pathways that transfer glutathione (GSH). In addition to transferring GSH, SNK-1 activates enzymes that serve as radical scavengers, and Nrf2 controls the phase 2 detoxification response. Studies by Guerrero-Rubio et al.[25-26] *in vivo* manifested that betanidin has a potent effect in comparison to other betalain against oxidative damage induced in *C. elegan*, and the expression of the above-mentioned regulatory pathway was identified. Moreover, the same studies explained that betalains—those with a cyclic amino acid and those that were negatively charged, such as betacyanin, betaxanthin, indicaxanthin, and dopaxanthin—were the most efficient against oxidative stress.

Three major betalain compounds (betanin, vulgaxanthin I, and indicaxanthin) affected the expression of Nrf2 signaling pathway enzymes and Nrf2-regulated catalytic glutamate-cysteine ligase catalytic subunit (GCLC) and glutamate-cysteine ligase modifier subunits, which support key enzymes in GSH biosynthesis[11]. Especially, betanin up-regulated *HO-1*, NADPH quinone oxidoreductase (*NQO-1*), *GCLC*, *GPX-1*, and glutathione S-transferase π-1 (*GSTP-1*) gene expression and most potently suppressed H_2O_2-stimulated ROS. Modulation of intracellular ROS caused by betalains is probably due to attenuated inflammation signaling factors and activation of Nrf2-signaling, which promotes transcription and the synthesis of proteins and enzymes involved in cellular redox regulation, such as HO-1 and NQO-1[11]. Investigation of the antioxidant effect of *S. huastecorum*, rich in betalains and indicaxanthin, demonstrated significantly higher Nrf2 protein levels, which indicate an antioxidative effect, and attenuated MDA levels, while no considerable change was observed in antioxidant enzyme activities and protein levels of SOD, CAT, and GPx[28]. Likewise, *Opuntia* fruit extracts that are rich in betacyanins, with betanin exhibiting the highest concentration, had distinct effects on antioxidant-related genes[27]. At the transcriptional level, it modulated the self-reliant expression of oxidative stress-related genes and prevented acetaminophen-induced *SOD-2*, *Hmox1*, and *Gclc* levels, *in vivo*, which were not dependent on ROS-scavenging activity[27]. All the above studies indicate the antioxidant effect of betalains, whereas the efficacy of compounds varies with type. In general, betacyanin is more effective than betaxanthin.

4. Anti-inflammatory activity of betalain

Pro-inflammatory cytokines and other inflammatory mediators make a substantial contribution to the progression of cell damage and functional impairment of tissues in diseased conditions. Betalains and its derivatives have been demonstrated to have multi-organ protective properties, beginning with the reduction of pro-inflammatory cytokines such as IL-1β, IL-6, IL-18, and TNF-α, which up-regulate inflammatory reactions (Table 1)[13]. An evaluation of the effect of beetroot juice, an excellent source of betacyanin and betaxanthin, on isoproterenol-induced myocardial injury in rats, suggested potent anti-inflammatory activity with alleviation of TNF-α, IL-6, and IL-10 levels together with substantially attenuated NF-κB DNA-binding activity[29]. Besides this, betalain attenuates the expression of inflammatory enzymes such as cyclooxygenase (cyclooxygenase-1 (COX-1)) (which is connected to prostaglandin

Table 1
Antioxidant and anti-inflammatory mechanism of betalains from different sources.

Model	Source/Doses provided	Activity	Investigations	Results	Reference
Caenorhabditis elegans	Dopamine/25 μmol/L	Antioxidant	Glutamic acid-6-decarboby-betaxanthin and glutamic acid-6-decarboby-betaxanthin administration	Reduction of oxidative stress and increased life span by 7% with anti-aging effect	[24]
C. elegans	Plant extracts or microbial/25μmol/L	Antioxidant	Age-synchronized worms are supplemented with betalains	Betalains influence the expression of aging genes through the DAF-16/FOXO and SNK-1/Nrf2 pathway	[25-26]
Wistar rats	*Opuntia* red-purple fruits (betacyanins)/800 mg/kg, orally	Antioxidant	Investigated liver damage biomarkers, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, lactate dehydrogenase, malondialdehyde (MDA), and mRNA expression of oxidative stress-related genes	Modulated oxidative stress-related genes expression at the transcriptional level	[27]
Caco-2 cell from colorectal adenocarcinoma	*Beta vulgaris* L. and *Opuntia ficus-indica* L.	Antioxidant, anti-inflammatory	Betanin, vulgaxanthin I and indicaxanthin, was applied to intestinal epithelial cells to analyze inflammatory and redox-related cell signaling effect	Transcription of proinflammatory mediators (cyclooxygenase-2 and inducible NOS (iNOS)), and enhance antioxidant enzymes (heme oxygenase-1 (HO-1)) secretion, mitigate oxidative stress in intestine cell	[11]
Wistar male rats	*Stenocereus huastecorum* fruit (betalains)/400 mg/day	Antioxidant, anti-inflammatory	Effect of administered pitaya juice concentrate on cisplatin-induced nephrotoxicity	Mitigate tissue damage and protect against oxidative stress and nephrotoxicity by nitric oxide (NO) pathways	[28]
Male Swiss	*B. vulgaris*/10–1 000 mg/kg	Antioxidant, anti-inflammatory	Examine the effect of betalains on abdominal contortions in mice	Attenuate tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β, lipid peroxidation, upregulated Nrf2 and NF-κB with HO1 transcript expression and superoxide anion levels	[13]
Wistar rats	*B. vulgaris* juice/ 150 and 300 mg/kg	Anti-inflammatory	Studied the healing effect of *B. vulgaris* juice on rats with myocardial injury	Reduced cardiac inflammatory cytokines (IL-6, TNF-α, and IL-10), NF-κB DNA binding, and protein expression of NF-κB (p65)	[29]
Sprague-Dawley rats	Betanin/10, 20 and 40 mg/kg	Anti-inflammatory	Administration of different doses of betanin in randomly selected diabetic rats	*NF-κB* mRNA expression was downregulated in comparison to diabetic control rats	[30]

stimulation), 5-lipoxygenase (5-LOX), and NADPH oxidase-1 (Nox-1)[111]. Additionally, it inhibits the secretion of other inflammatory cytokines and markers such as C-X-C chemokine receptor type 4 (CXCR4), and IL-17[11,31-32].

On exploration of the effect of beetroot extract, the levels of pro-inflammatory cytokinin-TNF-α and IL-6 in the culture cell were usually reduced, while in the liver tissue, it decreased the expression of NF-κB signaling pathway and TNF-α levels[30,33]. The combination of IL-1β and TNF-α is widely established as one of the primary inducers of the response to acute inflammation in cell lines[28]. Incubation with betanin, vulgaxanthin, and indicaxanthin in a cell line resulted in considerable inhibition of IL-6 and IL-8 expression, with indicaxanthin and vulgaxanthin efficiently suppressing IL-6 and IL-8 expression, respectively, at all doses[11]. This indicates that betanin, vulgaxanthin, and indicaxanthin could disrupt the NF-κB pathway in inflamed intestinal cells[11]. Likewise, Ramírez-Rodríguez et al.[28] reported that a crude extract of *S. huastecorum* fruit abundant in betalain minimized tissue damage and protected against oxidative stress and nephrotoxicity via NO routes. Indicaxanthin from different plant sources modulates macrophage redox state, repairs NF-κB-dependent damage, protects mitochondria from oxidative stress, and inhibits NOX-4 activity and expression[34].

5. Organ protective effect of betalains

Betalains are widely studied for various biological activities and health-promoting benefits (Table 2). Betalains feds to *C. elegan* showed reduced oxidative stress and activated the transcription factors DAF-16/FOXO and SKN-1/Nrf2 thereby increasing the longevity of the animal[25]. Additionally, this bioactive compound protects various human organs through antioxidant and anti-inflammatory activities (Fig. 2)[7].

Table 2
Organ-protecting ability of betalain via different mechanisms in the animal model.

Experimental subject	Dose of betalain	Health benefits/Outcome	References
Heart protection			
Adult male Sprague-Dawley rats	25 and 100 mg/(kg·day)	Betanin at both concentrations recovers isoproterenol-induced acute myocardial infarction in rats via inhibition or suppression of iNOS, inflammation, oxidative stress, and low-density lipoprotein (LDL) level	[35]
Sprague-Dawley rats	100 mg/kg	Betanin-administered rats showed a significant reduction in MDA, myeloperoxidase (MPO), and IL-6 levels in hearts compared to the control ischemia/reperfusion rats. Additionally, a decrease in the apoptotic cell was noted	[36]
Liver protection			
Sprague-Dawley rats	25 and 100 mg/(kg·day)	Betanin (25 and 100 mg/kg) significantly attenuates paraquat-induced liver injury in rats by lowering oxidative stress, ALT levels, and mitochondrial damage compared to the control animals	[37]
Wistar rats	20 mg/kg	Betanin feed rats showed significant improvement in antioxidant enzyme system, glucose level, and reduction in hepatica damage biomarkers such as ALT and AST levels	[18]
Kidney protection			
Male Sprague-Dawley rats	25 and 100 mg/(kg·day)	Betanin (25 and 100 mg/kg) alleviated the paraquat-induced kidney injury through the reduction of inflammation, NF-κB, and lysosomal protease activities. Also lowered serum and urine biomarkers for kidney injury by increasing the antioxidant system	[38]
Sprague-Dawley rats	8 mL/(kg·day)	The supplementation of beetroot juice to the nephrotoxicity-induced rats showed that the antioxidant system enhanced which lowered oxidative stress, and inflammation biomarkers in serum and kidney	[39]
Neuro protection			
Male ICR mice	50 and 100 mg/(kg·day)	Betanin enhances spatial learning and protects against neural death and neurotransmitter alteration	[40]
Parkinson male ICR mice	100 and 200 mg/kg	Betanin shows neuroprotective properties against mouse models of Parkinson's disease in terms of both motor impairment and neurodegeneration	[41]
Chronic constriction injury (CCI), male ICR mice	1−1 000 mg/kg	Betanin modulates microglial activation in a dose dependent manner during neuropathic pain	[42]
Alzheimer's disease Wistar rats	25 and 50 mg/kg	By lowering mitochondrial ROS, betanin improves scopolamine-induced memory loss, tissue damage, and mitochondrial dysfunction, as betanin has strong antioxidant properties	[43]
Male Wistar albino rats	25 mg/kg BW	Betanin reduces the harmful effects of disruption in brain neurotransmitters, making betanin an amazing neuroprotective antioxidant	[44]
Lung protection			
BALB/c mice	20, 60, and 180 mg/kg	Immunoglobulin E (IgE), eosinophil infiltration, eotaxin, and helper T cell (Th)2 were downregulated at dosages of 60 and 180 mg/(kg·day), whereas 180 mg/(kg·day) decreased percentages of cytotoxic T cell (Tc)17, Th17, and Tc2 as well as Th17- and Th2-signature cytokines	[14,45]
Charles River Wistar rats	50 mg/kg	Betanin protects the pulmonary tissues and reduces inflammatory cell density following intestinal ischeamia-reperfusion (IR) injury	[46]
Spraque-Dawley rats	100 mg/kg	Betanin with copper protected rats' hearts and lungs from IR injury via anti-inflammatory and antioxidant pathways	[36,47]
Nude mice	25 and 50 μmol/L	Betalain improves the expression of pro-inflammatory cytokines with suppressed toxicity	[48]
Gut protection			
Colitis BALB/cJ mice	1 g/kg	Degradation of proinflammatory cytokinin by betanin reduced inflammation and improved murine colitis	[17]
Charles River Wistar rats	50 mg/kg	Betanin protects the jejunal mucosa and reduces inflammatory cell density following intestinal IR injury	[46]
Repro protection			
Male albino rats	Different doses of red beetroot (RBR) extract	Betanin significantly attenuates cisplatin-induced testicular toxicity in rats by lowering MDA, with increasing GSH and CAT levels compared to the control animals	[49-50]
Wistar rats	300 mg/kg beetroot extract	Beetroot extract protects the testis by controlling hormone levels, preventing oxidative damage, and reducing inflammation	[20]

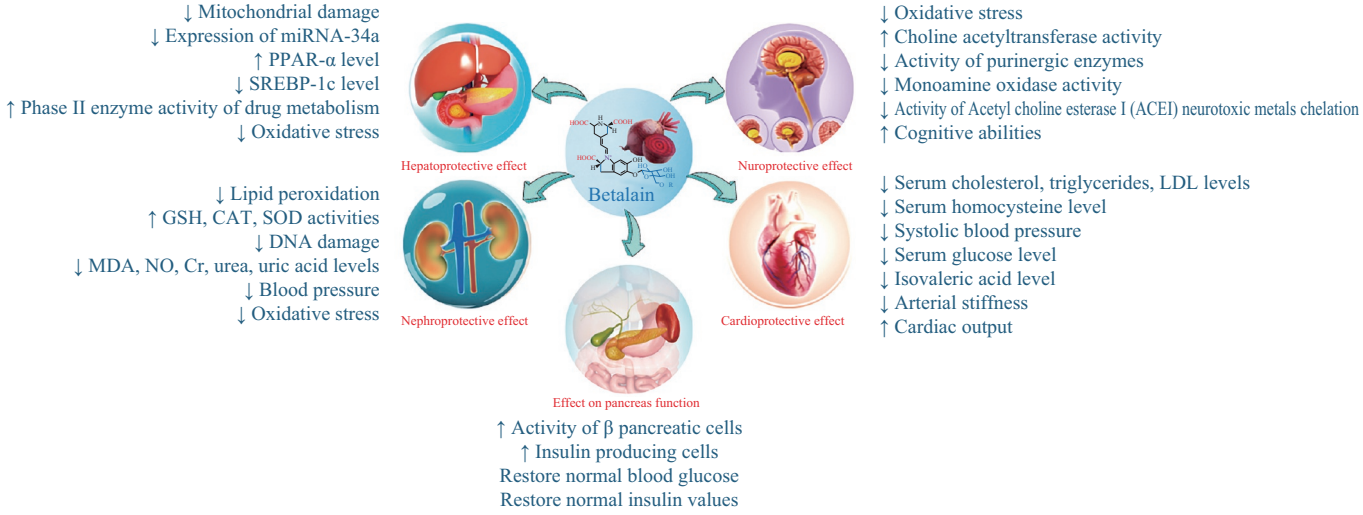

Fig. 2 Multiple organ protective effects of betalains and their derivatives. ↑, ↓. arrows indicate upregulation and down regulation, respectively.

5.1 Cardio-protective effect

The cardiovascular disease occurred due to a blockage of blood supply to cardiac muscle and the brain. This blockage can be mediated by peripheral arterial disease, cerebrovascular disease, and coronary heart disease[15]. Also, hyperlipidemia, obesity, diabetes, and hypertension are the major causes of worldwide human deaths and are considered the main risk factors for cardiovascular disease[51]. Several clinical studies have shown that beetroot juice supplementation successfully reduced high cholesterol, the lipid profile, and systolic blood pressure in adults[10,52]. However, most of the researchers claimed that the reduction of oxidative stress and the cholesterol- or hypertension-lowering effect of beetroot juice were related to the presence of nitrate[51,53-54]. Nevertheless, betalain is the major bioactive pigment presented in the beetroot. Hence, neumerous studies are inclined towards the effect of betalain on cardiovascular diseases[15]. In this context, the protective effect of betanin (25 and 100 mg/(kg·day)) against fructose (30%)-induced diabetic cardiac fibrosis in Sprague-Dawley rats was investigated for 60 days by Han et al.[55]. The rats fed only fructose showed significant diabetic cardiac fibrosis, as evidenced by increasing profibrotic factor transforming growth factors-β1 (PFTGF-β1) and decreasing soluble collagen content. On the other hand, betanin treatment significantly inhibited the formation of PFTGF-β1 and lowered cardiac collagen accumulation. Furthermore, betanin reduced protein glycation, oxidative stress, and NF-κB activation, thereby inhibiting cardiac fibrosis in fructose-fed rats[55]. Furthermore, by stimulating the Nrf2/HO-1 pathway, betanin inhibited the activation of toll-like receptor 4 (TLR4)/NF-κB signaling in the aorta wall and decreased the levels of tissue-derived ROS and circulating 8-isoprostane[56]. In another study, betanin (25 and 100 mg/(kg·day)) supplementation was investigated against isoproterenol (100 mg/kg)-induced acute myocardial infarction in adult male Sprague-Dawley rats[35]. The results indicated that betanin significantly improved cardiac structure and function in isoproterenol-treated rats by inhibiting inducible NOS, NF-κB activation, oxidative damage, and low-density lipoprotein levels[35]. Similarly, the beneficiary protective effect of betanin (100 mg/kg) on the hearts of ischemia/reperfusion-injured Sprague-Dawley rats was demonstrated by Tural et al.[36]. The authors reported that betanin treatment lowered MPO, MDA, IL-6, and apoptotic cells in the hearts of IR-injured rats. All the above studies suggested that betalain could support cardiac health by boosting the antioxidant enzyme system, increasing blood flow, and reducing inflammation, thereby enhancing myocardial function and structure.

5.2 Hepato-protective effect

The liver is an important body organ. It works to control lipid and carbohydrate stores. It also maintains an adequate supply of metabolites necessary for both intensive physical activity as well as muscle and brain tissue formation. It is also important for the excretion of harmful metabolites and hence susceptible to ROS generation which could increase the cell damage risk[37].

Beetroot juice may prevent liver damage through metabolic changes. In this context, Krajka-Kuźniak et al.[57] studied the hepatoprotection by beetroot juice in rats with *N*-nitrtosodiethylamine induced liver injury, and reported that the prolong feeding of beetroot juice increases the phase II enzyme activity thereby activating the Nrf2-antioxidant response element pathway. Also, the pretreatment of beetroot juice significantly decreased nitrtosodiethylamine induced increased ALT, gamma-glutamyl transferase (GGT), and succinate dehydrogenase activities. Furthermore, the same group evaluated the cytoprotective effect of betanin in human liver cell lines[58]. The results suggested that betanin exerts a hepato-protective effect on the non-tumor THLE-2 cell line through the activation of Nrf2 which was induced by mitogen-activated protein kinases. In another study, the organ protective effect of betanin was investigated on paraquat-induced liver toxicity in rats[37]. The rats were divided into 4 groups at random: a control group, a paraquat group, and two groups that received betanin at injections of 25 and 100 mg/(kg·day) 3 days before and 2 days after receiving paraquat. To study the organ protective effect of betanin on mitochondrial damage, enzymatic activities, histopathology, oxidative stress, and cytochrome P450 (*CYP*) *3A2* mRNA expression of the liver was assessed[37]. It was observed that the group of rats with induced liver injury found

histological alterations. The increased serum AST and ALT levels; oxidative stress, an elevated *CYP 3A2* mRNA expression, swelling of mitochondrial membrane, apoptosis-inducing factor proteins, and dropped mitochondrial cytochrome C were observed. Pathological damage and all of the above-mentioned markers were lower in betanin-treated animals than in paraquat-only animals. In rats, betanin protected paraquat-induced liver damage. Protecting the liver is done by stopping the expression of CYP 3A2 and protecting the mitochondrial wall[37].

Also, the RBR juice supplementation for 8 weeks (8 mL/(kg·day)) to the CCl_4 induced hepatotoxicity rats showed lowered inflammatory markers and reduced hepatic damage by 19%−26%. In addition, a high level of antioxidant enzymes, and hepatic enzymes was observed with reduced lipid peroxidation in beetroot juice-supplemented rats[59]. On the other hand, short-term supplementation of betanin (20 mg/kg) for 20 days showed a reduction of oxidative stress in Wistar rats[18]. The oxidative stress in rats was induced by feeding a hyperlipidemic diet for 60 days. The betanin feed rats showed a significant reduction in oxidative stress by increasing the antioxidant enzyme system and reducing MDA. Additionally, betanin reverses hepatic tissue damage by reducing ALT and AST levels as well as evidenced by histopathological analyses[18]. All these studies suggested that betalains could be considered therapeutic compounds to treat elevated oxidative stress and thereby protect against liver damage.

5.3 Renal protection

Tan et al.[38] examined the effect of betanin on paraquat-generated acute nephrotoxicity in rats. Investigation on 4 different groups of rats, a paraquat group, and two paraquat groups with betanin at doses of 25 and 100 mg/(kg·day) subjection 3 days before and 2 days following paraquat injection. Betanin inhibited paraquat-induced inflammation by reducing the expression of inducible NOS and cyclooxygenase, blunting activation of NF-κB, and decreasing lysosomal protease activity. Furthermore, betanin reduced the oxidative stress caused by paraquat. It was observed that betanin improves the paraquat-generated acute renal injury by improving the histological parameters, and reducing serum and urine markers of renal injury[38]. The effect of methanolic extract of RBR against *O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate (CPF)-induced nephrotoxicity in rats was evaluated[19]. This study evaluated the nephroprotective effects of RBR extract against chlorpyrifos-induced renal problems. CPF exposure increased serum creatinine, urea, and MDA levels while reducing antioxidant system and nuclear factor (erythroid-derived 2)-like-2 factor expression causing renal dysfunction. CPF also caused renal tissue inflammation and necrosis, as shown by elevated TNF-α, interleukin-1 release, Bax, and caspase-3 levels. Administration of RBR 1 h before CPF treatment blocked all biochemical changes that occurred due to CPF toxicity. The antioxidant, anti-inflammatory, and anti-apoptotic properties of RBR may be effective against CPF-induced nephrotoxicity[19]. In another study, Iahtisham Ul et al.[39] evaluated the nephroprotective effects of RBR-based beverages in rats with gentamicin-induced renal stress. Normal and nephrotoxicity-induced rats were administered beetroot-based beverages (8 mL/(kg·day)) for 8 weeks. Gentamicin (85 mg/(kg·day)) was used to induce renal stress in rats during the final week of the supplementation. Following that, overnight fasted rats were sacrificed, and their sera and kidneys were analyzed. It was observed that antioxidant enzymes in renal tissues and serum proteins were significantly improved, whereas lipid peroxidation, NO, urea, and creatinine levels were temporarily reduced. Furthermore, histological analysis revealed that rats given beet beverages had better renal parameters. The results suggest that drinks made with RBR could help reduce the ill effects of gentamicin-caused nephrotic stress. Overall, betalains could enhance the renal function and protect the kidney through antioxidant and anti-inflammatory activities.

5.4 Neuroprotective effect

Neurodegenerative diseases, including Alzheimer's and Parkinson's, are the current global challenges and are caused by disturbances of the neuronal complex. To understand it, insights into neuroinflammation, oxidative stress, neurotoxicity, endothelial dysfunction, and metabolic imbalance are most important[60]. To some extent, activation of pro-inflammatory cytokines and release of ROS via various pathways contribute to the aforementioned condition—for example, inflammation at the microglial and neuronal levels in Alzheimer's disease due to amyloid-β accumulation—resulting in a decrease in neurotransmitters and cognitive symptoms[61-62]. According to studies, betalains act as neuroprotective compounds because it reduces inflammation and oxidative stress caused by neurotoxicants[60], amyloid-β aggregation[61], mitochondrial dysfunction[43], microglia activation in the brain[62], thereby protecting against neurodegeneration[40].

To explain the anti-inflammatory and antioxidant properties of betalains over neurotoxicants, Di et al.[60] investigated the application of betalains in aluminum chloride-induced Alzheimer rats, and the result reported a diminution of MDA (lipid oxidation) through regulation of antioxidant enzymes like SOD, GSH, and CAT. Betalains alleviated the expression of pro-inflammatory cytokines—IL-6, IL-1β, COX-2, iNOS, and TNF-α—and signaling pathways (NF-κB) via mRNA expression reversion. Neurological degeneration by environmental and medicinal toxicity resulted in neurological lipid peroxidation (MDA) and oxidative stress was effectively ameliorated by the inhibition of MDA secretion, serum Nrf2 level, and promoting activity of antioxidative enzymes-CAT, GSH, and SOD[60]. Also, betanin helps to avoid spontaneously hypertensive-groups interaction with organotin as a free radical scavenger[40-41,44,63]. In another study, the effect of betalains and their derivatives on the accumulation of amyloid-β, a factor of neuroinflammation, was studied by Imamura et al.[61]. The results suggested that betalains and iso-betalains significantly attenuated amyloid-β-induced toxicity as the result of delayed paralysis in *C. elegans*. Neuronal impairment by amyloid β leads to mitochondrial dysfunction, and neuronal cells are noted to be very sensitive to mitochondrial dysfunction; their protection is the most important. Administration of betalains demonstrated the protection of mitochondria by attenuation of ROS formation[43].

Microglia activation, on the other hand, releases free radicals and inflammatory cytokines via NF-κB pathways with the assistance of Toll-like receptors, which implicates neurodegenerative progression[42]. Hereby, suppression of lipopolysaccharide-induced microglial activity is achieved through high negative binding affinity against active areas of TNF-α, NF-κB, NO, and IL-6, and inhibition of IL-1β, free radicals, particularly ROS/reactive nitrogen species (RNS)[42,62].

5.5 Lung protection

The lung facilitate the exchange of gases through the inhalation of oxygen and the exhalation of CO_2. While exposure to various environmental toxicants causes narrowing and clogging of airways, which can lead to clinical conditions such as asthma, pulmonary edema, airway inflammation with and without infection, and eventually malignancy. In addition to these, in cases of extremity ischemia and reperfusion, the lung is the most vulnerable organ, and inflammation-related complications can occur. Herein, betalains demonstrated the ability to overcome the aforementioned conditions both *ex vivo* and *in vivo*[14,36,48]. Li et al.[45] investigated whether dose-dependent treatment of betalains in ovalbumin-induced mice mitigates allergic asthma. Betalains attenuate the T-cell subunits, cytokinin levels, peripheral cell inflammation, upregulation of the cAMP-PKA-CREB pathway with specific protein expression, and recovery of the inflamed airways[45]. Furthermore, in an asthmatic condition, betalains ingestion resulted in a reduction in lung's weight due to mucosal drainage, inflammatory cell infiltration in bronchoalveolar lavage fluid, and decreased IgE and cytokine levels. In asthma-induced mice, it ameliorates lung mechanics by reducing oxidative stress, NO levels, TGF-β regulated gene expression and signaling transduction of Smad proteins[14].

During IR, multiple organs, including the lung, are damaged as a result of the ROS released by macrophages and neutrophils, inhibiting inflammation in the organ. In the case of a robust inflammatory response in lung tissue cells during intestinal ischemia, Toth et al.[46] examined the effect of parenteral betalains pretreatment in the intestinal IR rat and discovered that betanin has lung parenchyma-preserving properties. Evidence of pulmonary lesions with inflammation and alveolar bleeding was reported to have decreased, with a steady reduction in MPO and mast cell expansion in betalains pretreated rats. An experimental study showed the coupled ingestion of betalain and copper considerably reduced MDA and MPO levels in rats, which indicated the potential protective effect of betalains in the lung during IR[36]. Besides these, betalains are also effective against small lung cells. A study conducted in bronchial epithelial mice unveiled that the pro-inflammatory cytokinin (IL-6, TNF-α, and IL-1β) expression was markedly decreased, which suggested that betalains mitigate the inflammatory response during lung small cell carcinoma in athymic mice[48]. Further, on the lung's histopathology examination, infiltration of inflammatory cells in lung cell carcinoma was reported[48]. Through this, a clear insight can be gained into the fact that betalains and their derivatives have potent lung protection properties.

5.6 Gut-protective effect

Gastrointestinal diseases such as celiac disease, colitis, inflammatory bowel disease (IBD), and Crohn's disease are the major diseases associated with inflammation and intestinal line damage. Several cell and *in vivo* studies have shown that betalains have the potential to protect the stomach and intestine from disease-related inflammation and damage. Smeriglio et al.[64] investigated the impact of betalains-rich prickly pear treatment in the inflamed intestine and concluded that betalains have a promising healing effect. The extract inhibits the release of ROS and inflammatory mediators such as IL-6, IL-8, and NO. In another experimental study in murine colitis, the expression of mRNA of the pro-inflammation cytokines (TNF-α, IL-1β, COX-2, IL-6, and iNOS) and macrophage markers (CD11c, CCR7, and CD86) was attenuated[17]. Moreover, mitigation in the mucosal protein levels of a pro-inflammatory complex (cytosolic BCL10), NF-κB-p65, and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha, p indicate phosphorylated (p-IκBα) was noticed, while the anti-inflammatory cytokine (IL-10) expression was elevated considerably[17], indicating the intestine protective effect of betalains. Even in jejunal ischemia-reperfusion injury, betalains have demonstrated the potential for an anti-inflammation response. In rats, pretreatment with betalains before jejunal ischemia resulted in mast cell reduction, and suppression of the inflammatory response MPO, resulting in a lower reperfusion rate than previously observed with the protection of intestinal mucosa[46]. Thus, betalains has potential for utilization in nutraceuticals for gut health protection in a range of clinical conditions.

5.7 Repro-protective effect

Reproductive organs account for the production of gametes (eggs and sperm), sex hormones, and fertilization sites. A decrease in gamete production and other functional deformities can be induced by exposure to different toxicants. Repro-toxicity-associated inflammation and oxidative damage are likely to happen, whereby betalain and its derivatives have shown a protective effect towards such inflammation and free radical-related damage[20]. An investigation of the histopathology of cisplatin-induced testicular damage in rats and the healing effect of beetroot extract by Hassen et al.[50] identified a considerable reduction in IL-6, MDA (as ascribed to ROS surplus that attacks the cell membrane), *iNOS* gene mRNA levels, and slightly elevated GSH and total antioxidant capacity (TAC) in rats by beetroot extract. This indicates suppression of the inflammatory response cell and upregulation of antioxidant activity[49]. Similarly, Albasher et al.[20] observed that beetroot extract pretreatment reduced MDA, NO, IL-1β, TNF-α and IL-6, and *Nos2* mRNA, while boosting the Nrf2/ARE (antioxidant response element) pathway and scavenging activity in chlorpyrifos-exposed testicular tissue via NF-κB inactivation. To date, a dearth of research is available on the protective effect of betalains in reproductive tissues and organs, but this provides evidence of a potential anti-inflammatory and antioxidative effect in reproductive organs.

6. Conclusion

The health benefits of betalains have been intensively studied and reported. This review provided an in-depth discussion of recent research focused on the multi-organ protective effect of betalains and their derivatives via the antioxidant and anti-inflammatory pathways. This information can be utilized to understand how betalains and their derivatives upregulate the functionality of human organs during impairment. Also, this would provide the mechanism of betalains and their derivatives to downregulate the mediators of inflammation and oxidative stress, which can provide new insight to the pharmaceutical and nutraceutical industries. The several *in vivo* studies, especially in animal models, reported here offer a strong foundation for considering medical assertions and the multi-organ-protecting functions of

betalains in humans. Thus, the promising results outlined in this paper characterize betalains and their derivatives as having promising prospects in medical and functional food applications. Yet it is necessary to consider that animal studies do not completely reproduce human physiology. Unless sufficient exploration of the protective impact of betalains is done in the medical sector with a large population, this proven organ protective effect of betalains in cell lines and animal models is still confined to lab research. Therefore, it is recommended that the upcoming study focuses on the application of betalains and their derivatives, as well as their impact on the human population, to effectively understand the regulatory mechanisms of betalains via antioxidative and anti-inflammatory marker regulation.

Conflicts of interest

Baojun Xu is an associate editor for *Food Science and Human Wellness* and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests.

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