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REVIEW

Betalains Alleviate Exercise-Induced Oxidative Stress, Inflammation, and Fatigue and Improve Sports Performance: an Update on Recent Advancement

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Abstract

Purpose of Review Beetroot juice is a popular natural food supplement commonly consumed for its health and ergogenic benefits. It contains an abundance of phytochemical compounds, which have been shown to enhance sports endurance and recovery. Among them, nitrate is well-studied and known for improving performance during exercise. On the other hand, betalains, the bioactive pigment, have shown various biological activities including antioxidant, anti-inflammatory, and anti-hypertensive, which may improve exercise performance and post-exercise recovery. Additionally, free radical scavenging activities of betalains could increase nitric oxide availability in the blood, thereby improving blood flow and oxygen supply during strenuous exercise. This review article provides a critical discussion of the non-pathological conditions induced by prolonged or strenuous exercise and betalains' potential in reducing such conditions including muscle damage, inflammation, and fatigue. Additionally, the real-time application of betalains as an ergogenic compound in competitive athletes has been discussed. Finally, future directions and conclusions on the potential of betalains as a natural ergogenic aid in sport endurance are outlined.

Recent Findings Betalains in beetroot are the major water-soluble nitrogen-containing pigment possessing high antioxidant, anti-inflammatory, and anti-fatigue activities. Betalain supplementation could alleviate exercise-induced oxidative stress, inflammation, and fatigue in competitive athletes.

Summary Betalains have the potential to become a natural ergogenic aid or nutraceutical compound for sports people during exercise and competitive performance.

Keywords Betalains · Bioactivities · Ergogenic compound · Sports · Sport performance

Introduction

Athletes undergo extensive and high-intensity physical training programs to maximize performance [1], which has been shown to increase the production of reactive oxygen species [2] and markers of muscular damage, lipid peroxidation, and pro-inflammatory cytokines [3, 4•]. These exercise-induced physiological changes result in increased muscle soreness and reduced exercise capacity [5]. Additionally, lack of sufficient rest and/or recovery process, in conjunction with heavy training load, detrimentally affects the functional capacity of athletes and results in poor performance [1, 4•, 6••]. Nevertheless, marginal improvements in performance can translate into large gains in performance [7]. Consequently, athletes often consume nutritional supplements to alleviate adverse physiological changes and enhance recovery between bouts [8]. Generally, supplements are used by all competitive sports athletes during workout, training, and competitions, while elite athletes reported to consume more supplements compared to other sport athletes [8–10].

In recent years, the popularity of nutritional supplements has surged. This is partially due to their potential ergogenic

Statement of Significance This review provided the current research on betalains as an ergogenic aid for sport performance improvement. Literature suggests that betalains are a major compound in beetroot followed by nitrate and betalains possess various biological activities including antioxidant, anti-inflammatory, antihypertensive, anti-cancer, antimicrobial etc. Additionally, betalains may enhance the blood flow of the sports people during exercise and competitive performance. Betalains could enhance sports and exercise performance by boosting the antioxidant and anti-inflammatory systems as well as eliminating toxic metabolites.

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properties, which have been shown to improve athletic performance and recovery [10, 11]. Nutritional supplements can be foods, such as fruits and vegetables, herbs, amino acids and proteins, vitamins, and n-3 polyunsaturated fatty acid (n-3 PUFA) [4•, 5, 12]. In general, all nutritional supplements for sports performance have been differentiated based on scientific evidence, nature, potency, as well as regulation and legislation of each country [9, 11]. In comparison to chemical supplements, food or food products do not require harsh safety and regulatory evidence [11]. Therefore, most athletes choose organic food containing active compounds or food supplements to enhance performance during training and competitions [13, 14]. Beetroot is one of the well-known and extensively studied vegetables as a nutritional aid for competitive athletes [7, 13, 15, 16]. Beetroot is reported to be an excellent source of minerals, nitrate, phenolics, carotenoids, ascorbic acid, vitamins, amino acids, and betalains [17, 18, 19•]. Among the bioactive compounds present in the beetroot, betalains (606.3 mg/100 g dry matter) [17, 20] and nitrate (64.4–180 mg/100 g dry matter) [21] are the most abundant compounds.

The effects of dietary nitrate in beetroot on exercise endurance are well-studied and reported for various sports performance [7, 13, 22]. Upon ingestion, the dietary nitrate is reduced to nitric oxide through various metabolic actions [21, 23]. This nitric oxide has hemodynamic and metabolic functions, which have been shown to increase blood flow to working tissue, improve mitochondrial efficiency, and enhance muscle contraction [22, 24]. Besides nitrate in beetroot, betalains, beetroot's bioactive pigment, are also reported to improve blood flow and enhance health and performance, albeit through different mechanisms [25••]. Betalains appear to possess numerous biological activities such as antioxidant, anti-inflammatory, anti-cancer, and anti-hypertensive effects [15, 25••, 26]. However, the application of betalains as an ergogenic aid is limited. Therefore, the purpose of this review article is to explore the biological activities of betalains related to sports endurance such as antioxidant, anti-inflammatory, and anti-fatigue. In addition, betalains in sports performance and future directions for the use of betalains as an ergogenic aid are discussed.

Betalain Structure

Betalains are water-soluble nitrogen-containing pigments with betalamic acid as a core structure to all betalain pigments [26]. Betalains consist of two structural groups: red-violet betacyanins and yellow betaxanthins [27•]. The complete betalain biosynthesis pathways start from tyrosine to betalamic acid, and further formation of betaxanthin or betacyanin has been briefly explained by Hatlestad et al. [28] and Khan and Giridhar [29]. The major betacyanin pigments reported are betanin, isobetanin, and neobetanin, while vulgaxanthin I, vulgaxanthin II, and indicaxanthin are the major betaxanthin pigments present in the beetroot (Fig. 1) [17, 19•].

Exercise-Induced Pathological/ Non-pathological Conditions and Betalain Biological Activities

Exercise-Induced Oxidative Stress

Oxidative stress occurs due to the imbalance of pro-oxidant and antioxidant defense systems in favor of the former [30]. While regular exercise is known to improve well-being by reducing various stress-related damages to the body, it has been reported that prolonged and intensive exercise produces an excessive amount of reactive oxygen species (ROS) and free radicals, which can negatively impact performance [31]. Generally, normal physiological levels of ROS are required for optimal muscle force production [2], whereas high levels of ROS result in contractile dysfunction and thus fatigue [32]. It is well known that during exercise, oxygen demand increases in the skeletal muscle. However, intensive exercise dramatically changes the physiological conditions in muscle fibers making them produce higher concentrations of ROS via (a) mitochondria, (b) phospholipase A2 (PLA2), and (c) nicotinamide adenine dinucleotide phosphate (NADPH) oxidase pathway [33]. Nevertheless, present research indicates that mitochondria and PLA2 are not the primary sources of ROS production during exercise but rather NADPH oxidases (NOX) such as NOX2 and NOX4 [34, 35]. Among the two isoforms of NOX, NOX2 is responsible for the overproduction of ROS during exercise, whereas NOX4 contributes to the normal ROS production in muscle fiber [36]. Therefore, NOX2 in skeletal muscle is the major source of ROS during exercise [37]. ROS is a term used to describe highly reactive derivatives of molecular oxygen and includes superoxide radical (O_2), hydroxyl radical (HO), hydrogen peroxide (H_2O_2), and singlet oxygen (O^-) [38]. Other sources of ROS

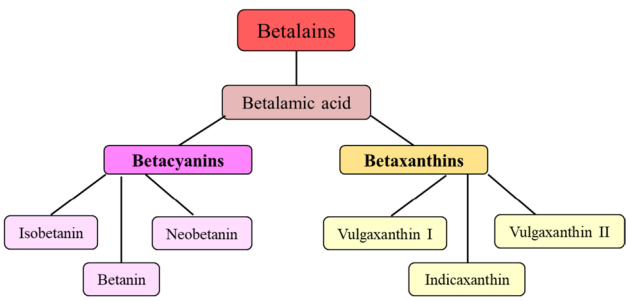

Fig. 1 Betalains and their class

are immune cells (e.g., macrophages, monocytes, and neutrophils) and enzymatic reactions (cytochrome P-450, peroxisomal oxidase, and xanthine oxidase) [2, 33, 37].

Overproduction of ROS consequently attacks cellular macromolecules, including DNA, proteins, and lipids, and subsequently damages various tissues and organs, which can lead to diseases (Fig. 2) [37]. Though not pathological, exercise-induced oxidative stress can reduce performance by disturbing several of the physiological processes required to perform the exercise task and maintain homeostasis. Exercise-induced oxidative stress can be measured by various biomarkers such as oxidants (ROS, reactive nitrogen species), antioxidants (total antioxidant capacity, glutathione, etc.), oxidation products (malondialdehyde, protein carbonyls, nitrotyrosine, etc.), and antioxidant/oxidant ratio (reduced glutathione/oxidized glutathione, thiol/disulfide state, etc.) [32, 39]. Although reactive nitrogen species (RNS) such as peroxy-nitrite are also produced during strenuous exercise, they do not play a major role in muscle contractile dysfunction or fatigue [33]. Exercise-induced oxidative stress, its cellular mechanism, and the increase in oxidative biomarkers have been extensively reviewed and reported earlier [30, 32, 39].

Antioxidant Activity of Betalains

Several plants, fruits, vegetables, and spices are well known for their antioxidant activities through various radical scavenging activities, metal chelators, enzyme inhibitory activity, and different cellular mechanisms [2, 40, 41]. Antioxidant activities of betalains have been evaluated and reported to be a class of dietary cationized antioxidants [42]. Betanin, the major bioactive pigment in red beetroot, contains phenolic and cyclic amine groups with strong electron donating ability [43, 44]. The health benefits of betalains that could potentially support the exercise-induced oxidative stress and inflammatory response are presented in Table 1.

The effects of betanin on ROS production and DNA damage in stimulated human polymorphonuclear neutrophils were investigated [45]. The authors reported that betanin (2–500 μM) inhibited ROS production and DNA damage in stimulated neutrophils in a dose-dependent manner. Thus, betanin could counterbalance the oxidative metabolism in neutrophils [45]. In another study, Krajka-Kuźniak et al. [46] investigated the effect of betanin in the liver cell line and found that betanin stimulates the nuclear factor erythroid-2 related factor (Nrf2), a major transcription factor that expresses numerous antioxidant genes, thus, increasing endogenous antioxidant capacity. Similarly, Esatbeyoglu et al. [47] examined the effect of betanin (25–500 μM) on the antioxidant enzyme system and DNA damage in Huh 7 human liver hepatoma cells. The result indicated that betanin dose-dependently inhibits hydrogen peroxide-induced DNA damage. Further, it induced the antioxidant enzyme system (heme oxygenase-1,

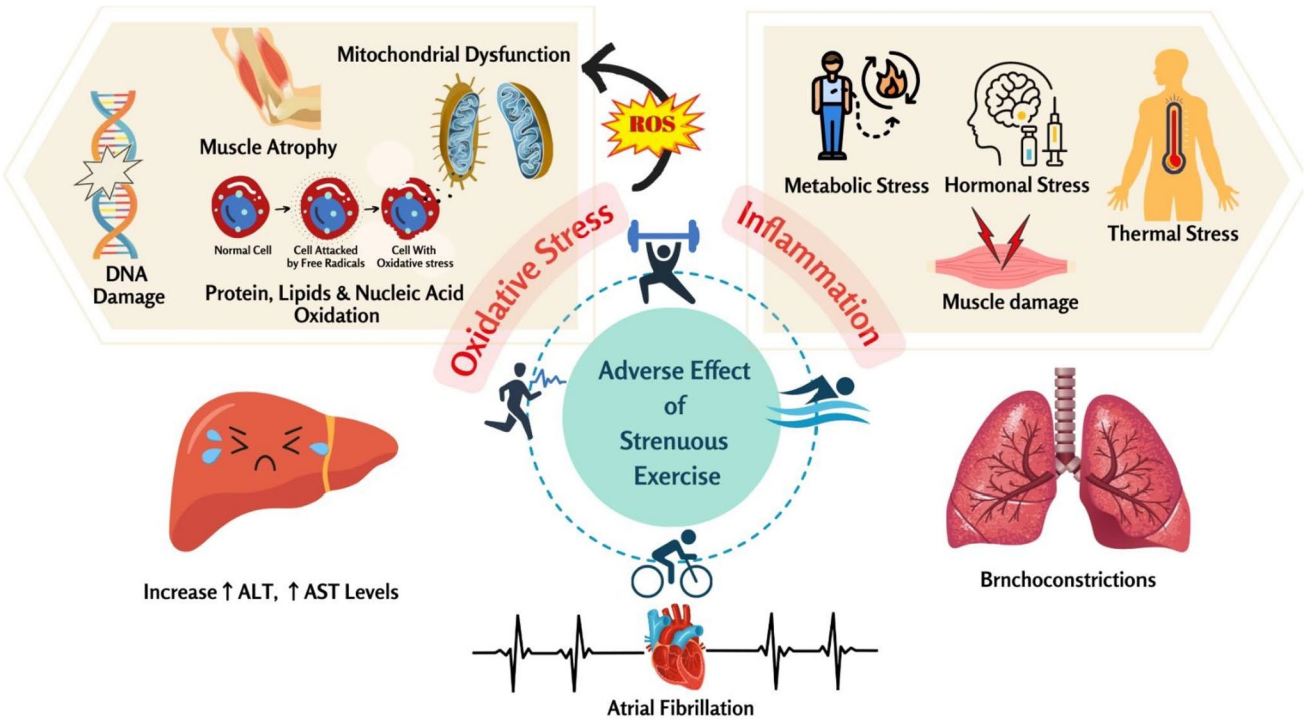

Fig. 2 Exercise-induced non-pathological and pathological conditions in athletes

Table 1 Bioactivities and health benefits of betalain that could support during exercise-induced non-pathological conditions

Experimental subject	Dose of betalain	Health benefits/outcome	References
Antioxidant activity			
Human polymorphonuclear neutrophils	2–500 μM	Betanin significantly inhibited the ROS production and also protected DNA damage induced by phorbol 12-myristate 13-acetate. The effect of betanin was dose dependent.	[45]
Free radical scavenging activity by electron spin resonance spectroscopy and Huh7 human liver hepatoma cells	10–100 μM for DPPH assay 50–500 μM for oxidase system 15 μM for cell assay	Betanin scavenges free radicals in a dose-dependent manner. In addition, betanin induced transcription factor Nrf2 and increased oxidative enzyme system in the cell line and thereby inhibits DNA damage.	[47]
Caenorhabditis elegans	20 μM	Betalains, namely, indicaxanthin, indoline carboxylic acid-betacyanin, phenylalanine-betaxanthin, and dopaxanthin, have shown strong antioxidant activities in various antioxidant systems. Additionally, they reduced the oxidative stress in *C. elegans* by activating the transcription factors DAF-16/FOXO and SKN-1/Nrf2.	[48]
Anti-inflammatory activity			
Male Swiss mice	100 mg/kg	Betalain treatment significantly reduced pre- or post-carrageenan-induced edema. In addition, betalain significantly reduced total leukocytes, superoxide radicals, TNF-α, and IL-1β level in the peritoneal fluid. On the other hand, betalain enhances anti-inflammatory (IL-10) levels.	[57]
Lipoxygenase and cyclooxygenase enzyme system	Varies with different betalains and enzymes	Phenethyl-amine-betaxanthin at 125 μM was post-potent to inhibit cyclooxygenase, whereas lipoxygenase was inhibited by betanidin and indoline derivative of betalain with IC_{50} of 41.4 and 40.1 μM, respectively.	[61]

paraoxonase-1, and glutathione) by activating Nrf2 factor [47]. Han et al. examined the effect of betanin on paraquat-induced liver toxicity in rats [44]. Betanin (25 and 100 mg/kg body weight/day) was fed to rats 3 days prior and 2 days following paraquat ingestion. The researchers reported that betanin protected the rats from liver failure by augmenting the antioxidant enzyme system, cytochrome P450, and mitochondrial damage in rats compared to the rats without betanin ingestion [44]. Recently, the antioxidant capacity of betalains (25 μM) was investigated in an animal model using *Caenorhabditis elegans* [48]. The researchers found that different types of betalains had different effects on the inhibition of oxidative stress via increasing antioxidant system and the transcription factors DAF-16/FOXO and Nrf2. For example, indicaxanthin, indoline carboxylic acid-betacyanin, phenylalanine-betaxanthin, and dopaxanthin increased lifespan of *Caenorhabditis elegans* by 16.82%, 16.65%, 16.53%, and 12.93%, respectively [48]. Based on these studies, betalains may attenuate oxidative stress by enhancing endogenous antioxidant capacity via the transcription factor Nrf2, thereby reducing oxidative stress and DNA damage.

Exercise-Induced Inflammation

Inflammation is a complex biological response of the body to harmful external stimuli or stressors. This can be described by a series of cellular reactions leading to capillary dilatation, production of blood-borne soluble components, and an increase in body temperature [49]. This pro-inflammatory response can be induced during and after the acute exercise, which can extend beyond the exercise task. It is well known that this pro-inflammatory response is important for subsequent adaptation; however, if gone unabated could have negative impacts on performance and recovery [50]. The pro-inflammatory response is largely dependent on the exercise intensity, the type of muscle contraction, and/or the novelty of exercise [49]. Cytokines, immune cells, and other intracellular components interact in the skeletal muscle during and after acute or strenuous exercise, thereby creating an inflammatory milieu responsible for recovery and adaption [50]. Eccentric-biased exercise is known to cause ultrastructural damage to skeletal muscle tissue. In response, the body immediately coordinates an immune response, mobilizing leukocytes, mainly neutrophils, to the damaged

tissue to break down and clear the damaged proteins [51]. In addition, local macrophages (M1) are activated to assist in the removal of cellular debris. In the following days, a macrophage phenotype transitions from pro-inflammatory M1 to anti-inflammatory M2 macrophages promoting the regenerative process [51]. Generally, the release of pro-inflammatory cytokines such as interleukin-6 induces free fatty acid consumption for the increase in energy, neutrophil mobilization, and activation of anti-inflammatory cytokine (interleukin-10 and interleukin-1 receptor antagonist (IL-1 ra)) for recovery [52]. However, excessive release of pro-inflammatory cytokine during strenuous or unaccustomed exercise enters into the blood circulation which creates more damage to healthy tissues [53]. Several gene polymorphisms including angiotensin I-converting enzyme (ACE), α-actinin skeletal muscle isoform 3 (ACTN3), creatine kinase (CK), insulin-like growth factor 2 (IGF2), myosin light chain kinase (MLCK), interleukin-6 (IL-6), and the tumor necrosis factor (TNFα) were involved in exercise-induced muscle damage [54]. Besides this surge in ROS results in oxidative damage of surrounding tissue and myocyte (muscle cells), which have been traced to a majority of exercise-induced inflammation [52], ROS generated during strenuous exercise might initiate a series of redox-sensitive intracellular signaling events. Hollander et al. [55] reported that exhaustive exercise activates activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB), which are transcription factors involved in inflammation. Similarly, the interaction between exercise-induced ROS and inflammation transcription factors was documented by Gomez-Cabrera et al. [56].

Anti-inflammatory Activity of Betalains

The antioxidant activities of betalains make it a suitable treatment for inflammation. Betalains have shown promising potential in the treatment of inflammation-associated diseases. Martinez et al. [57] investigated the anti-inflammatory properties of a betalain-rich dye of *Beta vulgaris* against ROS, leukocyte recruitment, edema, and cytokine production in carrageenan-induced paw edema in mice. The authors reported a significant reduction in tumor necrosis factor-alpha (TNF-a), carrageenan-induced superoxide anion, and IL-1b levels in the peritoneal fluid in mice, while IL-10 levels were increased when subjected to betalain treatment. The increased anti-inflammatory cytokine IL-10 production induced by betalain is noteworthy as there is much evidence on the capability of IL-10 in retarding NF-κB activation via the inhibition of TNF-α and IL-1b production [58]. Furthermore, a reduction in TNF-α, IL-6, and growth-regulated protein alpha (GRO-α) was observed in 10 osteoarthritic patients treated with capsules produced from beetroot extract (particularly at dosages greater than 35 mg) delivered twice daily orally for 10 days [59]. Similarly, inflammatory responses (IL-6, TNF-α, MPO, and NF-κB) were decreased in 24 albino Wistar rats fed with ethanolic extract of beetroot (250 and 500 mg/kg) for 20 days [60]. The author claimed the anti-inflammatory effect of the extract was due to the presence of betalains. Additionally, Vidal et al. claimed that betalains suppressed the inflammatory response by attenuating cyclooxygenase and lipoxygenase activity [61]. Betalains inhibit these two enzymes by interacting with specific amino acids at the active site pocket of the enzyme, forming a stable complex that renders the enzyme inactive [61].

Exercise-Induced Fatigue

Fatigue is a complex phenomenon defined as the inability to sustain muscular work at a given intensity [62]. Manifestations of fatigue can range from perceived tiredness, exhaustion, or lack of energy which subsequently reduces physical activity and performance [63]. The origins of exercise-induced fatigue can occur centrally (i.e., in the central nervous system) or peripherally, considered to occur at or beyond the neuromuscular junction (Fig. 3) [64]. Disturbances in redox hemostasis generally occur at higher exercise intensities. The excess production of ROS has been shown to depress muscular force in both human and animal models. ROS can affect skeletal muscle function in several ways, primarily by oxidizing proteins responsible for calcium (Ca2+) handling and the myofibrils themselves, and reducing myofibrillar Ca2+ sensitivity. In addition, ROS are implicated in vascular and mitochondrial dysfunction and muscle damage [65], which have collectively been linked to fatigue.

Anti-fatigue Activity of Betalains

Supplements such as polysaccharides, amino acids, proteins, vitamins, and phytochemicals can combat exercise-induced fatigue by providing instant energy (glucose, amino acids, or proteins), acting as antioxidants (phytochemicals or peptides), or delaying the onset of neurotransmitter formation (branch chain amino acids or phytochemicals) [62]. Among them, betalains have been well-studied for their antioxidant and anti-inflammatory activities in different in vitro and in vivo model studies. The bioactivities of betalains were discussed in the above sections "Antioxidant Activity of Betalains" and "Anti-inflammatory Activity of Betalains". Betalains have a strong ability to scavenge ROS, boosting the antioxidant enzyme system as well as recovering mitochondrial damage in living organisms [44, 46]. Also, betalains have been shown to reduce inflammatory responses (IL-6, TNF-α, MPO, and NF-κB) and enhance anti-inflammatory cytokine IL-10 [59, 60]. Similarly, other fruit juices including blueberries [66] and tart cherry [67] have been reported to increase exercise performance by enhancing total antioxidant status thereby reducing

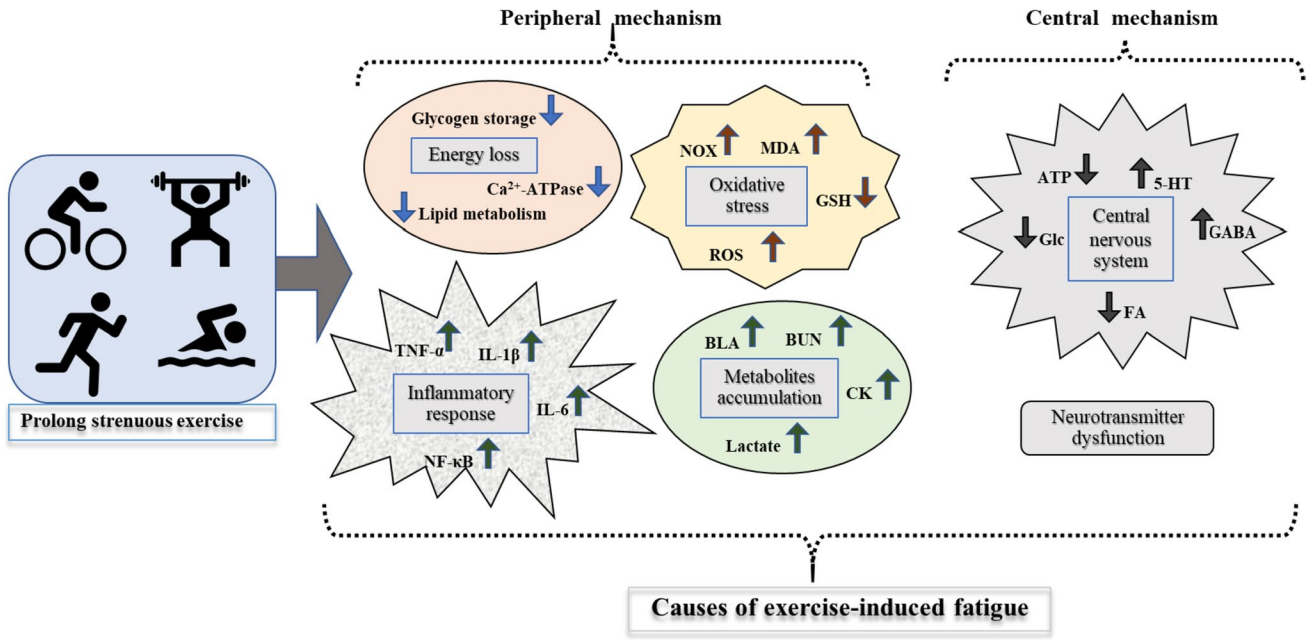

Fig. 3 Causes of exercise-induced fatigue in athletes

inflammatory factors and fatigue. Recently, Liu et al. [64] reviewed various traditional Chinese medicines utilized in relieving exercise-induced fatigue. Authors suggested that phytochemicals including polyphenols, flavonoids, and terpenes are the major bioactive components in traditional medicines. Also, these compounds exhibit anti-fatigue activities via various mechanisms such as improvement of energy metabolism, removal of metabolites, boosting antioxidant and anti-inflammatory activities, and regulating neurotransmitter formation [64]. Overall, the anti-fatigue activity of betalain could be related to the versatile biological activity of betalains, which could help to maintain the antioxidant balance, mental wellness as well as neurohormonal balance after post-exercise.

Betalains and Sport Performance

Betalains could be a promising compound to enhance sports performance owing to their numerous bioactivities. To date, only three studies have examined the benefits of betalains on exercise performance. Van Hoorebeke et al. [68] and Montenegro et al. [69••] evaluated the effect of betalain-rich concentrate juice (devoid of sugars and nitrate) of beetroot supplementation on exercise-related performance, muscle damage, and recovery in competitive runners and triathletes, respectively. In the first study, 13 competitive male runners were involved in double-blind, randomized two trials with supplementation of 100 mg betalain-rich concentrate or control for 6 days, separated by a week [68]. Additionally, on day 7, betalain-rich concentrate or control (50 mg) was supplemented to the runner before 150 min of exercise. The exercise consisted of 30-min treadmill running followed by a 5-km time trial competitive running. Betalain-rich concentrate supplementation was associated with lowered blood lactate concentration (14%), rate of perceived exertion (15%), and heart rate (3%) compared to the control in competitive runners. In addition, fast performance was reported in 10 out of 13 runners during 5-km time trial competition [68]. Thus, betalain-rich concentrate supplementation for competitive runners may improve time trial performance while controlling muscle soreness, fatigue, and muscle damage. Montenegro et al. examined the effects of a similar dosing scheme of betalains on exercise performance and recovery in a group of 22 triathletes [69••]. The exercise tasks consisted of 40 min of cycling performed for 2 h following 50 mg of a betalain-rich concentrate or control supplementation followed by a 10-km competitive running. In addition, to assess recovery performance, participants performed a 5-km time trial 24 h later. The outcome of the study indicated that betalain-rich concentrate supplementation did not lower the average heart rate or perceived exertion among participants. However, faster 10-km time trial performance was observed in the betalain concentrate group. The betalain group also exhibited an attenuated rise in creatine kinase, a marker of muscle damage, following the 10-km time trial. Five-kilometer

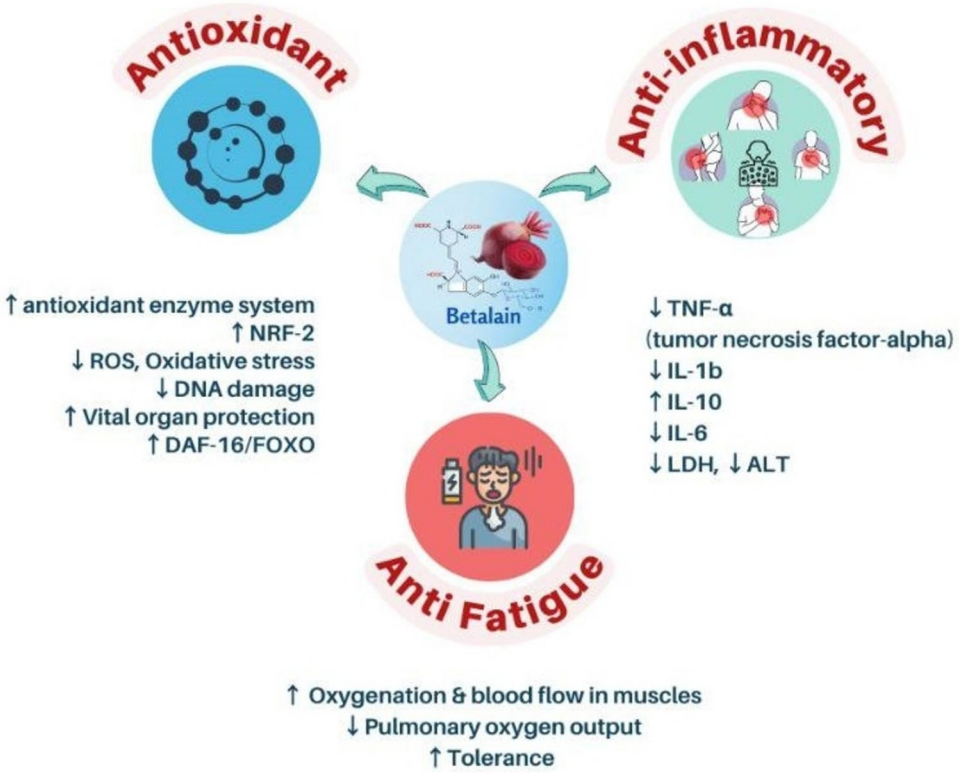

Fig. 4 Betalains in augmenting exercise-induced oxidative stress, inflammation, and fatigue effect

time trial recovery performance was faster following betalain-rich concentrate compared to the control [69••]. Overall, betalain-rich concentrate supplementation may enhance performance and recovery following exercise [69••]. Mumford et al. [6••] investigated the effects of a similar betalain supplement on the cycling performance of well-trained male cyclists using a similar dosing strategy as the aforementioned studies. On the last day of supplementation, cyclists performed 30-min cycling time trial. The blood samples were obtained from all participants before and following time trial cycling to determine various biomarkers. Also, the exercise performances including heart rate, beat-by-beat arterial blood pressure, stroke volume and cardiac output, oxygen uptake, and respiratory gas exchange ratio were measured following the cycling time trial. Betalain-rich concentrate supplementation was associated with improved the average absolute power and exercise efficiency of cyclists compared to the placebo group. No other differences were observed in the inflammatory markers, oxidative stress, pH, lactate, and plasma nitrite (NOx) between groups [6••]. Interestingly, the betalain-rich supplementation improved performance in the last 5 min of the cycling time trial as well as blood flow without an increase in inflammation or oxidative stress. Hence, betalain supplementation could act as a natural ergogenic aid for the enhancement of sports/exercise performance (Fig. 4).

Future Direction and Conclusion

Although the research investigating the ergogenic benefits of betalains is limited, the aforementioned studies suggest the potential application of betalains as an ergogenic agent in competitive athletes. However, more research with larger sample sizes is warranted. In addition, future studies should focus on the effect of various doses of betalain supplementation on the sportsperson's age, gender, and other physical condition. Further, biochemical analyses of the blood samples of the subjects should be conducted such as oxidative stress levels, inflammatory levels, oxygen levels in the blood, and organ performance. Also, the effect of betalain should be conducted on eccentric and strenuous exercise in elite athletes and their recovery potential. Lastly, betalain purified and isolated from beetroot should be examined for its high dose toxicity and adverse effect on any organ of the sportsperson to validate the safety issue.

In conclusion, strenuous exercise can induce disturbances in redox homeostasis and increase inflammation, which can negatively impact performance. Betalains possess a wide spectrum of biological activities including antioxidant and anti-inflammatory properties. Athletes undergo strenuous and rigorous exercise before and during the competitive performance, which subsequently induces chronic oxidative stress, inflammation, and fatigue. Hence, providing the betalain supplement before or in between bouts may help

to reduce oxidative stress, inflammation, and subsequently fatigue in athletes. Also, betalains could enhance post-exercise recovery by stimulating the antioxidant enzyme system, improving blood flow, reducing inflammatory biomarkers, and reducing muscle damage. Hence, betalains could be a new ergogenic compound for competitive athletes. Although there are lots of reports on various biological activities and health benefits of betalains, further, gastrointestinal stability and pharmacokinetics, and toxicity of betalains need to be studied.

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Author Contribution The authors' contributions were as follows: SN, OOO, and SM searched and collected literature, compiled data, and drafted some parts of the manuscript. NPN conceived the work, searched and collected literature, drafted, revised, and edited the manuscript. SV and CK revised and edited the manuscript.

Compliance with Ethical Standards

Ethical Approval All authors are agreed to for their accountable contributions and approval of the manuscript.

Conflict of Interest The authors report no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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