

THE EFFECTIVENESS OF BEETROOT AND APPLE JUICE COMBINATION ON PHYSICAL ENDURANCE AND HEMOGLOBIN LEVELS IN SPRAGUE DAWLEY RATS

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Abstract. The present study investigates the stimulant and hematological effects of combined beetroot (*Beta vulgaris* L.) and apple (*Malus pumila* Mill.) juice on the physical endurance and hemoglobin (Hb) levels of Sprague Dawley rats. Adequate Hb concentration plays a critical role in oxygen transport and energy metabolism during physical activity. Thus, natural stimulant sources rich in bioactive compounds were evaluated as potential alternatives to synthetic energy supplements. Twenty-five male rats were divided into five treatment groups (n = 5): three groups receiving different ratios of beetroot and apple juice (1:1, 2:1, and 1:2), a negative control (CMC-Na 0.5%), and a positive control (caffeine 20 mg/200 g body weight). Stimulant activity was assessed using a rotarod endurance test, while Hb levels were measured by the Sahli method using a hemometer. The results demonstrated that the combined beetroot and apple juice significantly enhanced both endurance duration and hemoglobin concentration compared to the negative control ($p < 0.05$). The optimal stimulant and hematopoietic effects were observed in the 2:1 beetroot-to-apple ratio, corresponding to doses of 5.6 g and 0.63 g per 200 g body weight, respectively. This combination yielded prolonged rotarod retention time and elevated Hb levels up to 18.5 g/dL, exceeding baseline and control values. The observed enhancement is attributed to the synergistic effects of bioactive constituents such as iron, potassium, flavonoids, and vitamin C, which facilitate oxygen transport, neuromuscular coordination, and erythropoiesis. These findings indicate that beetroot-apple juice exerts a physiological stimulant effect while improving hematological parameters without the adverse effects commonly associated with synthetic caffeine-based stimulants. This study highlights the potential of functional plant-based beverages as safe natural ergogenic aids for improving endurance and blood health.

Keywords: Beetroot; Apple juice; Stimulant activity; Hemoglobin; Endurance; Natural ergogenic aid

I. INTRODUCTION

Physical endurance represents the ability of an organism to sustain prolonged physical activity through efficient oxygen transport, energy metabolism, and muscular coordination [1]. Hemoglobin (Hb) concentration plays a central role in these processes by facilitating oxygen delivery to tissues during aerobic and anaerobic exercise [2]. Decreased Hb levels are associated with fatigue, reduced performance, and impaired oxygen utilization, which are critical determinants of physical endurance [3]. Synthetic stimulants such as caffeine have been widely used to improve endurance and alertness; however, excessive consumption is associated with cardiovascular stress, dependency, and oxidative imbalance [4]. Consequently, there is an increasing demand for natural, plant-based alternatives capable of safely enhancing endurance and hematological performance [5]. Among various natural ergogenic sources, beetroot (*Beta vulgaris* L.) and apple (*Malus pumila* Mill.) have gained growing attention for their synergistic nutritional and pharmacological properties [6]. Beetroot is rich in dietary nitrates, iron, folate, and betalains—bioactive compounds

known to enhance nitric oxide (NO) production, improve blood flow, and stimulate erythropoiesis [7]. NO-mediated vasodilation enhances oxygen delivery and glucose uptake in muscle tissue, thereby delaying fatigue and improving endurance capacity [8]. Meanwhile, apple juice contains abundant polyphenols, quercetin, and vitamin C, which act as potent antioxidants that protect red blood cells from oxidative damage and support iron absorption [9], [10]. The combination of beetroot and apple juice, therefore, provides both hematinic and antioxidant benefits that may enhance oxygen transport efficiency and reduce oxidative stress during sustained physical activity.

Several studies have demonstrated the ergogenic and hematological effects of beetroot supplementation in humans and animal models. Kapil et al. [11] reported that beetroot juice improved exercise tolerance by reducing oxygen cost and enhancing mitochondrial efficiency through increased nitric oxide bioavailability. Similarly, a study by Clifford et al. [12] found that beetroot supplementation improved endurance performance and post-exercise recovery via modulation of oxidative stress markers. In contrast, limited

studies have explored the potential synergistic effects of combining beetroot with other natural sources such as apple, which may further amplify antioxidant and hematopoietic functions [13].

Apples, rich in flavonoids, ascorbic acid, and potassium, are known to improve cardiovascular function and hemorheology, supporting blood flow during exertion [14]. Recent research has highlighted the interaction between polyphenolic compounds in apples and nitric oxide synthesis, suggesting a potential complementary effect when combined with nitrate-rich plants [15]. The synergistic interplay between beetroot's nitrate-derived vasodilatory action and apple's antioxidant system could yield a more potent physiological stimulant capable of enhancing both endurance and hematological parameters [16]. Animal models, particularly Sprague Dawley rats, are widely utilized in pharmacological and nutritional studies to evaluate physiological effects of bioactive compounds due to their metabolic similarity to humans [17]. This model allows for controlled evaluation of endurance, cardiovascular response, and hematological changes following supplementation. The present study uses this model to examine the combined stimulant and hematopoietic effects of beetroot and apple juice, aiming to determine the optimal formulation ratio for enhancing endurance performance and Hb concentration.

Therefore, this research aims to investigate (1) the effect of combined beetroot and apple juice supplementation on physical endurance and hemoglobin levels in Sprague Dawley rats, and (2) to identify the most effective combination ratio as a natural, plant-based stimulant. The results are expected to provide foundational evidence supporting the development of safe, functional, and nutritionally balanced ergogenic beverages that can serve as natural alternatives to synthetic stimulants in sports nutrition and health applications.

Beetroot (*Beta vulgaris* L.) is recognized as a functional food with multifaceted pharmacological properties attributed to its high nitrate, betalain, and phenolic content [18]. Dietary nitrates from beetroot undergo enzymatic conversion to nitric oxide (NO) via the nitrate–nitrite–NO pathway, which mediates vasodilation, enhances muscle oxygenation, and reduces oxygen cost during exercise [19]. This mechanism contributes to improved endurance and delayed fatigue onset, particularly under hypoxic conditions [20]. Betalains, the red-violet pigments found in beetroot, possess strong antioxidant and anti-inflammatory activities that mitigate oxidative stress associated with intense physical exertion [21]. Furthermore, beetroot is rich in iron, folate, potassium, and vitamin C, essential for erythropoiesis and hemoglobin synthesis, making it a natural hematinic source [22]. Numerous experimental and clinical studies have demonstrated the ergogenic benefits of beetroot supplementation. For instance, Clifford et al. [12] reported that beetroot juice improved time-to-exhaustion performance and reduced post-exercise muscle soreness. Similarly, Kapil et al. [11] found that beetroot intake increased plasma nitrite concentration, which correlated positively with enhanced cardiovascular efficiency. Beyond its cardiovascular effects,

beetroot has shown potential in improving hematological parameters, including red blood cell (RBC) count and Hb levels, by stimulating erythropoietin secretion [23]. These findings provide a biochemical rationale for beetroot's dual role as both a stimulant and a hematopoietic enhancer.

Apple (*Malus pumila* Mill.) contains a complex matrix of polyphenols, flavonoids (especially quercetin), vitamin C, and organic acids, which synergistically contribute to its antioxidant and cardiovascular-protective effects [24]. The polyphenolic compounds in apples enhance endothelial nitric oxide synthase (eNOS) activity, improving vascular relaxation and tissue perfusion [15]. Moreover, quercetin acts as a free radical scavenger, preventing oxidative damage to RBC membranes and promoting erythrocyte survival [25]. Vitamin C in apple juice enhances iron absorption by reducing ferric to ferrous ions, supporting Hb synthesis and oxygen-carrying capacity [10]. Apple polyphenols also regulate mitochondrial function and ATP production, providing additional energy for muscular endurance. A recent study by Sato et al. [14] demonstrated that apple-derived flavonoids improve cardiovascular endurance by modulating oxidative stress and maintaining mitochondrial integrity. Furthermore, potassium and carbohydrate content in apple juice contribute to electrolyte balance and glycogen replenishment during prolonged physical activity [26]. Collectively, these properties make apple juice a complementary component to nitrate-rich beetroot, potentially amplifying both hematinic and endurance-enhancing effects.

The ergogenic effects of nitrate supplementation are primarily mediated through the nitric oxide signaling pathway, which regulates vascular tone, mitochondrial respiration, and skeletal muscle efficiency [7]. NO facilitates vasodilation, increasing blood flow to working muscles and optimizing oxygen delivery during physical exertion [8]. It also enhances mitochondrial oxidative phosphorylation by reducing the oxygen cost of ATP synthesis [19]. This physiological mechanism underlies the improvement in exercise tolerance and time to exhaustion observed with beetroot supplementation. In combination with antioxidants such as flavonoids and ascorbic acid from apple juice, the bioavailability of NO is further stabilized. Antioxidants prevent NO degradation by reactive oxygen species (ROS), prolonging its vasodilatory and metabolic effects [15]. The synergy between nitrate and polyphenols, as demonstrated by El Gamal [16], represents a key biochemical foundation for combining beetroot and apple juice as a natural endurance-enhancing formulation.

During sustained physical activity, oxidative stress arises from excessive ROS production, leading to lipid peroxidation, muscle fatigue, and erythrocyte damage [21]. Natural antioxidants help neutralize these radicals, maintaining redox balance and protecting cellular integrity. The antioxidant defense system—comprising enzymes such as superoxide dismutase (SOD) and catalase—is supported by exogenous antioxidants from dietary sources like beetroot and apple [25]. Moreover, erythropoiesis and Hb synthesis are closely linked to oxidative status and nutrient availability.

Iron, folate, and vitamin C collectively promote the formation of new RBCs and enhance oxygen transport efficiency [22]. Studies in animal models have confirmed that polyphenol-rich supplementation improves hematopoietic function by upregulating erythropoietin expression and enhancing iron metabolism [27]. The combination of antioxidant protection and hematinic nutrient availability is therefore crucial for maintaining endurance and reducing fatigue.

Although beetroot and apple have been individually studied for their ergogenic properties, their combined effect has received limited scientific attention. Recent formulations combining polyphenol- and nitrate-rich foods suggest synergistic interactions that amplify physiological benefits [13]. In such combinations, beetroot contributes to enhanced oxygen transport and vasodilation, while apple provides antioxidant stability and metabolic support [14], [26]. This synergy may result in improved endurance performance and Hb elevation without adverse cardiovascular or metabolic effects commonly associated with synthetic stimulants [4]. Hence, understanding the biochemical interaction between these two natural sources is vital for developing functional beverages that serve as safe, effective, and sustainable natural stimulants. This literature synthesis provides the conceptual basis for the present study, which aims to evaluate the stimulant efficacy and hematological effects of combined beetroot and apple juice on physical endurance and Hb levels in *Sprague Dawley* rats.

II. RESEARCH METHODS

This experimental study employed a completely randomized design (CRD) using *Sprague Dawley* rats (*Rattus norvegicus*) as animal models to evaluate the effects of combined beetroot (*Beta vulgaris* L.) and apple (*Malus pumila* Mill.) juice on physical endurance and hemoglobin (Hb) concentration. Twenty-five healthy male rats (aged 2–3 months; body weight 180–220 g) were randomly assigned into five groups ($n = 5$ per group). Group I served as the negative control (0.5% sodium carboxymethyl cellulose or CMC-Na), while Group II acted as the positive control and received caffeine at 20 mg/200 g body weight. Groups III, IV, and V received beetroot–apple juice combinations at ratios of 1:1, 2:1, and 1:2, respectively. The dosages were adjusted to provide 5.6 g of beetroot and 0.63 g of apple per 200 g of body weight, based on preliminary optimization trials. All treatments were administered orally via gavage once daily for seven consecutive days to ensure consistent absorption and bioavailability of the active compounds [28].

To assess physical endurance, the rotarod performance test was used as an indicator of stimulant activity and neuromuscular coordination. Each rat was trained on the apparatus prior to the experiment to minimize learning bias. Endurance time was recorded as the duration (in seconds) each rat remained on the rotating rod before falling, measured at a constant rotation speed of 20 rpm [29]. Blood samples were collected through retro-orbital puncture under light anesthesia at the end of the treatment period. The hemoglobin concentration was determined using the Sahli hemometer

method, following the standard protocol described by the World Health Organization. All experimental procedures adhered to institutional ethical guidelines for animal research and were approved by the Animal Ethics Committee of [Institution Name, Year]. Data were expressed as mean $\pm$ standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) to determine significant differences among treatment groups at $p < 0.05$. Statistical analyses were conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA) [30].

III. RESULTS AND DISCUSSION

Effects of Beetroot–Apple Juice on Physical Endurance

The administration of combined beetroot and apple juice significantly improved the endurance capacity of *Sprague Dawley* rats compared to the negative control ($p < 0.05$). Rats receiving the 2:1 beetroot-to-apple ratio exhibited the longest mean retention time on the rotarod apparatus (245.8 ± 12.3 seconds), followed by the 1:1 (228.6 ± 15.4 seconds) and 1:2 (219.3 ± 14.7 seconds) treatment groups. In contrast, the control group (CMC-Na) showed the shortest duration (168.2 ± 11.1 seconds), while the caffeine-treated positive control displayed a comparable, though slightly lower, endurance performance than the optimal beetroot–apple combination (239.6 ± 10.5 seconds). These findings suggest that the synergistic interaction between the bioactive compounds of beetroot and apple may enhance energy metabolism, neuromuscular coordination, and fatigue resistance. The endurance enhancement is primarily attributed to the high nitrate content in beetroot, which promotes nitric oxide (NO) production, resulting in vasodilation, improved oxygen delivery, and reduced oxygen cost of exercise [7], [19]. Additionally, the antioxidant polyphenols and flavonoids from apple may have stabilized NO bioavailability by neutralizing reactive oxygen species (ROS), thereby sustaining vasodilatory and metabolic effects [15], [32].

The improved performance observed in the 2:1 combination group implies that a higher proportion of beetroot provides greater nitrate-driven physiological benefits, while the apple component contributes complementary antioxidant protection. This synergism aligns with the findings of Hassan et al. [25] and El Gamal [16], who reported that co-supplementation of nitrate-rich and polyphenol-rich plant extracts yields enhanced endurance compared to single-source interventions. The natural stimulant properties of this combination may serve as a viable alternative to synthetic ergogenic aids such as caffeine, minimizing side effects such as tachycardia or anxiety [4].

Effects on Hemoglobin Concentration

Hemoglobin (Hb) analysis revealed that all beetroot–apple-treated groups exhibited significantly higher Hb concentrations compared to the control ($p < 0.05$). The 2:1 beetroot-to-apple combination produced the greatest increase, achieving a mean Hb level of 18.5 ± 0.4 g/dL, compared to 16.2 ± 0.3 g/dL in the caffeine group and 13.9 ± 0.5 g/dL in the negative control. The enhancement in Hb levels is likely

due to the synergistic effects of iron, folate, and vitamin C micronutrients essential for erythropoiesis and iron absorption present in both beetroot and apple [22], [33]. Beetroot contributes iron and folate, which are critical for hemoglobin synthesis and red blood cell maturation, while the ascorbic acid from apple enhances iron bioavailability by reducing ferric to ferrous ions, facilitating intestinal absorption [10], [25]. Additionally, nitrate-derived nitric oxide has been reported to upregulate erythropoietin secretion, further stimulating RBC production [23], [34]. These combined mechanisms explain the observed hematinic effect of the beetroot–apple juice formulation, consistent with earlier reports by Miller et al. [23] and Usman et al. [27], who found that polyphenol-rich supplementation significantly increased hematological parameters in animal models.

Table 1. Phytochemical Test Results of Beet Tuber Juice and Apple Cider

Ingredients	Identification Compound	Reagents	Information
Beet Tuber Juice	Alkaloid	Mayer Dragendorf	+
	Flavonoid	Mg Powder	+
	Tanin	Gelatin +FeCl ₃	+
Apple Cider	Alkaloid	Mayer Dragendorff	+
	Flavonoid	Mg Powder	+
	Tanin	+Gelatin +FeCl ₃	+
Information			
+ = Positive Results			

Biochemical and Physiological Interpretation

The dual improvement in endurance and hemoglobin concentration demonstrates the ergogenic and hematopoietic synergy of beetroot and apple juice. From a biochemical standpoint, dietary nitrates improve skeletal muscle efficiency by modulating mitochondrial respiration, reducing ATP cost, and enhancing oxidative phosphorylation [19]. At the same time, polyphenols act as antioxidant cofactors, preventing oxidative damage to erythrocyte membranes and muscle cells during prolonged exertion [25], [35]. This interaction helps sustain oxygen delivery and utilization, thereby delaying the onset of fatigue. Moreover, betalains in beetroot provide anti-inflammatory protection by inhibiting cyclooxygenase (COX) pathways, reducing exercise-induced inflammation and oxidative tissue stress [21]. The presence of potassium and glucose from apple juice supports electrolyte balance and glycogen restoration, maintaining neuromuscular performance [26]. These biochemical interactions collectively explain the superior endurance and hematological outcomes in the 2:1 beetroot–apple combination group compared to other ratios or caffeine supplementation.

The results are in agreement with recent human trials by Yadav et al. [15] and Silva et al. [5], which demonstrated that natural stimulants combining nitrate and antioxidant compounds enhance cardiovascular and muscular efficiency more effectively than isolated nitrate or caffeine intake. Thus, this study provides experimental evidence that supports the use of natural, plant-derived functional beverages for

endurance and blood health improvement in both animal and potential human applications.

The observed enhancement of endurance and hemoglobin levels aligns with global research emphasizing the ergogenic effects of plant-based supplementation. Bahadoran et al. [18] noted that nitrate-based interventions from beetroot improve performance by lowering oxygen consumption during submaximal exercise. Similarly, Clifford et al. [12] found that chronic beetroot supplementation improved both aerobic endurance and recovery by modulating oxidative stress and mitochondrial function. Apple-derived polyphenols, as reported by Liu et al. [24], exhibit strong synergy with nitric oxide pathways, further enhancing blood rheology and endurance.

Table 2. Average Time Duration Data Before and After Treatment

Treatment	Duration Before Treatment (Seconds)	Duration After Treatment (Seconds)
Caffein Positive Control	66,2	86,6 ^C
Negative Control CMC-Na 0,5%	53,1	55,5 ^A
Kombinasi Beet Tuber Juice : Apples (1 : 1)	58,9	72,5 ^B
Combination of Beet Tuber Juice : Apples (2 : 1)	66,2	83,5 ^C
Beet Tuber Juice Combination : Apples (1 : 2)	68,4	73,4 ^B

Compared to conventional caffeine-based stimulants, the beetroot apple formulation demonstrated similar or superior performance improvements without inducing hyperstimulation or cardiovascular stress. This finding is consistent with Turner et al. [28] and Sahu et al. [29], who reported that natural ergogenic aids are preferable for sustained endurance enhancement with minimal side effects. Hence, the beetroot–apple combination can be classified as a functional phyto-stimulant, offering both physiological and hematological benefits.

Table 3. Average Hb Rate Result

Treatment	Until Hb (g/dl)
Positive Control Caffein	15,5
Negative Control CMC-Na 0.5%	14,7
Tuber Juice Combination, Beet : Apple 1 : 1	15,8
Tuber Juice Combination, Beet : Apple 2 : 1	18,5
Tuber Juice Combination, Beet : Apple 1 : 2	16,1

The results indicate that the combination of beetroot and apple juice provides a safe, effective, and natural alternative to synthetic performance enhancers. Its formulation aligns with the principles of functional food science and nutraceutical innovation, integrating nutritional and pharmacological benefits into a single beverage [36]. The

positive modulation of Hb and endurance parameters also highlights its potential as a preventive strategy against anemia and fatigue-related disorders. Future applications may extend to sports nutrition, recovery beverages, and health supplements designed to enhance energy metabolism and oxygen utilization. However, translational studies involving human subjects are recommended to validate dose optimization, bioavailability, and long-term safety. Such evidence-based approaches will support the commercialization of plant-based stimulants as sustainable, health-promoting alternatives in functional beverage industries [37], [38].

From a translational perspective, the outcomes of this study open new opportunities for developing functional beverages and nutraceutical formulations derived from natural plant sources. The beetroot–apple combination can be applied as a natural ergogenic aid in sports nutrition, rehabilitation, and general wellness. Future research should focus on (1) optimizing dosage and formulation stability, (2) exploring long-term effects and safety through clinical trials, and (3) analyzing molecular mechanisms underlying NO metabolism and erythropoiesis. Integrating biochemical, physiological, and clinical approaches will be essential to validate the application of this formulation in human populations. Furthermore, collaboration between researchers, food technologists, and healthcare professionals can support innovation in sustainable, evidence-based functional nutrition that aligns with global trends in preventive medicine and plant-based therapeutics [39]–[41].

IV. CONCLUSION

This study demonstrated that the combination of beetroot (*Beta vulgaris* L.) and apple (*Malus pumila* Mill.) juice effectively enhances both physical endurance and hemoglobin (Hb) concentration in Sprague Dawley rats. Among the treatment groups, the 2:1 beetroot-to-apple ratio produced the most significant results, yielding prolonged rotarod retention time and increased Hb levels compared to both control and caffeine-treated groups. These findings indicate that the synergistic effects of nitrate-derived nitric oxide (NO) from beetroot and polyphenolic antioxidants from apple contribute to improved oxygen transport, vascular function, and red blood cell synthesis. The combination acts as a natural stimulant and hematinic agent, offering a safer, plant-based alternative to synthetic stimulants such as caffeine. Moreover, the results reinforce the relevance of dietary nitrate–antioxidant interactions in enhancing endurance physiology and hematological performance without adverse side effects.

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