3-29-2021

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Ashish Joseph
Christ College, India

Svenia P. Jose
St. Thomas College, India

Bintu T. Kalyan
Christ College, India

Renny R. Mammen
Akay Natural Ingredients Pvt. Ltd., India

I. M. Krishnakumar
Akay Natural Ingredients Pvt. Ltd., India

See next page for additional authors

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Digital Object Identifier (DOI)
https://doi.org/10.1016/j.nfs.2021.03.002

Notes/Citation Information
Published in *NFS Journal*, v. 23.

Authors
Ashish Joseph, Svenia P. Jose, Bintu T. Kalyan, Renny R. Mammen, I. M. Krishnakumar, Bradley S. Fleenor, and Ratheesh Mohan

NFS Journal 23 (2021) 37–43

Contents lists available at ScienceDirect

NFS Journal

journal homepage: www.elsevier.com/locate/nfs

Coconut inflorescence sap enhances exercise performance and plasma antioxidant status in young active men

Ashish Joseph [a], Svenia P. Jose [b], Bintu T. Kalyan [a], Renny R. Mammen [c], I.M. Krishnakumar [c], Bradley S. Fleenor [d], Ratheesh Mohan [b,*]

[a] Department of Physical Education, Christ College, Thrissur, Kerala, India
[b] Department of Biochemistry, St. Thomas College, Kottayam, Kerala, India
[c] R&D Centre, Akay Natural Ingredients Pvt. Ltd., Cochin, Kerala, India
[d] Department of Kinesiology and Health Promotion, University of Kentucky, Lexington, KY 40506, USA

ARTICLE INFO

Keywords:
COCOZEN
Endurance
Aerobic
VO_2 max
Peak power

ABSTRACT

Purpose: Nutrition has been increasingly recognized as a key component to optimal sports performance. Though several botanical agents have been reported to possess ergogenic potential, there exists a great interest for tasty and safe natural substances as performance boosters. In the present contribution, the ergogenic potential of a novel powder form of coconut inflorescence sap (CSP) was investigated for the first time.

Method: Out of the fourteen participants recruited, twelve recreationally active men completed the single-blinded, placebo-controlled, crossover study for 8 weeks. Running based anaerobic sprint test (RAST) and 2.4 km running test were performed as anaerobic and aerobic tests, respectively. In arm 1, the participants were received with either placebo (200 mL water containing 400 mg aspartame/day) or CSP (3 g in 200 mL water/day) for 21 days. After the washout period, arm 2 was performed with a reversed treatment regime. VO_2 max was estimated using a predictive formula.

Results: The primary outcome showed a significant enhancement in peak power and mean power (peak power from 3.67 W/kg b. wt. to 5.38 W/kg b. wt.; mean power from 3.47 W/kg b. wt. to 5.06 W/kg b. wt.). A significant ($p < 0.001$) increase in VO_2 max among CSP condition compared to the placebo was observed (from 59.38 ± 2.15 mL/kg/min to 62.56 ± 0.52 mL/kg/min). Further, serum analysis revealed enhanced antioxidant status and reduced lactate dehydrogenase ($p < 0.01$) levels without any significant changes ($p > 0.05$) in safety parameters.

Conclusion: It was concluded that CSP possesses significant ergogenic effect and may find wide application as a natural ingredient for sports nutrition and energy drinks.

Trail Registration: The study was registered in Clinical Trial Registry of India (Reg No.: CTRI/2018/03/012551 dated 13/03/2018).

1. Introduction

Nutrition is increasingly recognized as a key component to optimal physiological performance and function. Improving exercise performance with dietary modifications and/or supplementation with micro- and macro-nutrients has been on-going for nearly 100 years [1,2]. The development of both carbohydrate-enhanced beverages and foods as well as functional foods or nutraceuticals have been examined as potential ergogenic aids. Many of these dietary and supplemental approaches, however, are limited in use due to side effects such as gastrointestinal discomfort, lack of efficacy or the potential for adverse health consequences [3,4]. Thus, the development of novel and efficacious interventions to alter dietary micro- and macro-nutrients without causing side effects, yet leading to improved exercise performance are timely.

Coconut inflorescence sap tapped from the unopened flower buds of

Abbreviation: ALT, Alanine Aminotransferase; ALP, Alkaline Phosphatase; ANOVA, Analysis of Variance; AST, Aspartate Aminotransferase; BMI, Body Mass Index; CAT, Catalase; CSP, Coconut inflorescence sap; FBS, Fasting blood sugar; GPx, Glutathione Peroxidase; GI, Glycemic Index; Hb, Hemoglobin; HDL, High–Density Lipoprotein; LDH, Lactate Dehydrogenase; LDL, Low-Density Lipoprotein; ROS, Reactive Oxygen Species; RAST, Running-based, Anaerobic Sprint Test; SD, Standard Deviation; SEM, Standard Error of the Mean; SOD, Superoxide Dismutase; TC, Total Cholesterol; TG, Triglycerides; RNS, Reactive Nitrogen Speceis.

* Corresponding author at: Department of Biochemistry, St. Thomas College, Pala, Kottayam, Kerala, India.
E-mail address: biochemistrystcp@gmail.com (R. Mohan).

https://doi.org/10.1016/j.nfs.2021.03.002
Received 9 September 2020; Received in revised form 26 March 2021; Accepted 26 March 2021
Available online 29 March 2021

coconut trees (*Cocous nucifera L*) is a popular nutritious drink in Asian countries [5]. Coconut sap is rich in micro- and macro-nutritients, with various vitamins and minerals [6,7]. The processing of coconut sap, however, has been shown to damage the micronutrients limiting the use of this substance as a supplement. Recently, a micronutrient enriched Coconut Sap Powder (CSP) prepared by a low-temperature process, has shown hepatoprotective and nephroprotective effects both *in vitro* and *in vivo,* which was associated with improved antioxidant properties [5,8]. These data collectively suggest, CSP may be a novel supplement to enhance performance without resulting in any potential health-related side effects.

The purpose of this study, therefore, was to determine the efficacy of the novel, micro- and macro-nutrient rich CSP supplement on exercise performance in young, trained adults. We hypothesized that 3 g per day of CSP for 21 days will enhance anaerobic sprint power, and running performance in young, apparently healthy participants.

2. Materials and methods

2.1. Characterization of CSP

Proteins, fat and carbohydrates content of CSP were estimated by standarded procedures as per the official methods of Analysis of AOAC International [9]. Moisture, water activity and ash content were also estimated by the methods of AOAC [9]. Bulk density was determined as per the method of USP 29 (616). Vitamins (B2, B3, B6, B12 and C) were estimated by HPLC method as described by Sami et al., using Shimadzu LC 20 AT instrument fitted with an M20A photodiode array detector (PDA) (Shimadzu Analytical Pvt. Ltd., Mumbai, India) [10].

The elemental analysis was performed as follows. About 3 g of sample was accurately weighed into a crucible and subjected to ashing in a furnace for 4 h at 550 °C. After cooling in desiccator, 2.5 mL of 6 N HNO_3 was added to the crucible and the solution was filtered. The filtrate was made up to 100 mL with distilled water and was analyzed for Ca, Fe, P, Zn, Mn, Na and K by using Atomic Absorption Spectrophotometer (AAS-Perkin Elmer, Model analyst 800) [11].

2.2. Subjects

Healthy young male volunteers ($N = 14$) from Christ College, Kerala, India (Age 20 ± 1.5 years ; BMI 22.99 ± 0.79 kg/m^2) undergoing regular physical training for running, volleyball, swimming and jumping for at least 2–3 h/week were screened for the study. The small sample size can be rationalized on the basis of the fact that this is a pilot clinical study to investigate the efficacy of a micronutrient enriched CSP in endurance. Inclusion criteria were age (18–25 years), physical activity (a moderate activity with minimum 2 h of endurance exercise twice per week and a maximum of 3 h of endurance exercise thrice per week) with no history of musculoskeletal injury, pain or symptoms. Exclusion criteria were any current medical issues or history related to cardiovascular, liver, kidney or respiratory disease. Individuals taking herbals or other ergogenic supplements were excluded. Volunteers were asked to avoid caffeine-based drinks including coffee, tea or energy drinks during the study period. After detailing all the study procedures and risks, written informed consent was obtained from each participant. The study followed the protocol approved by the registered ethical committee (Reg. No ECR/184/Indt/KA /2014, dated 15/07/2014) and was conducted in accordance with the guidelines of Clinical Trial Registry of India (Reg No.: CTRI/2018/03/012551 dated 13/03/2018).

2.3. Study design and materials

A single-blinded (participants were blinded), placebo-controlled, randomized, cross-over design to consume either placebo (200 mL drinking water flavored and sweetened with 400 mg aspartame) or CSP (3 g CSP dissolved in 200 mL drinking water) as single dose per day, 30 min prior to breakfast for 21 days with a 2-week washout period between trials was adopted in this study (Fig. 1). Micronutrient-enriched coconut inflorescence sap powder (CSP, patent-pending and registered formulation as COCOZEN®) undergoing low-termperature processing and the placebo were prepared and provided by Akay Natural Ingredients, Cochin, India. A detailed analytical test report on various safety parameters including heavy metals, aflatoxins, microbial status, and pesticides was also received from the manufacturer. White crystalline powder of CSP was stored in a double-layered polyethylene bags under sealed conditions and kept at room temperature (25 ± 2 °C) in an air-tight high-density polyethylene container. Nutritional analysis for carbohydrates, proteins and fat along with various vitamins and minerals were performed as per the validated and approved analytical methods. Subjects were randomly divided into the placebo or CSP condition and were instructed to take the supplement daily for 21 days.

Following screening (Visit 1), participants underwent familiarization of the exercise protocols. During visit 2 (Day 1; Fig. 1) participants were instructed to wear comfortable, loose-fitting clothing and arrived at the physical education and sport sciences training center by 5 ± 0.5 PM. After 15 min of rest, blood samples (approximately 10 mL) were collected for baseline analysis followed by the exercise protocols (see below). All the physical tests were then performed in the same order and an additional ~10 mL of blood was collected immediately after exercise.

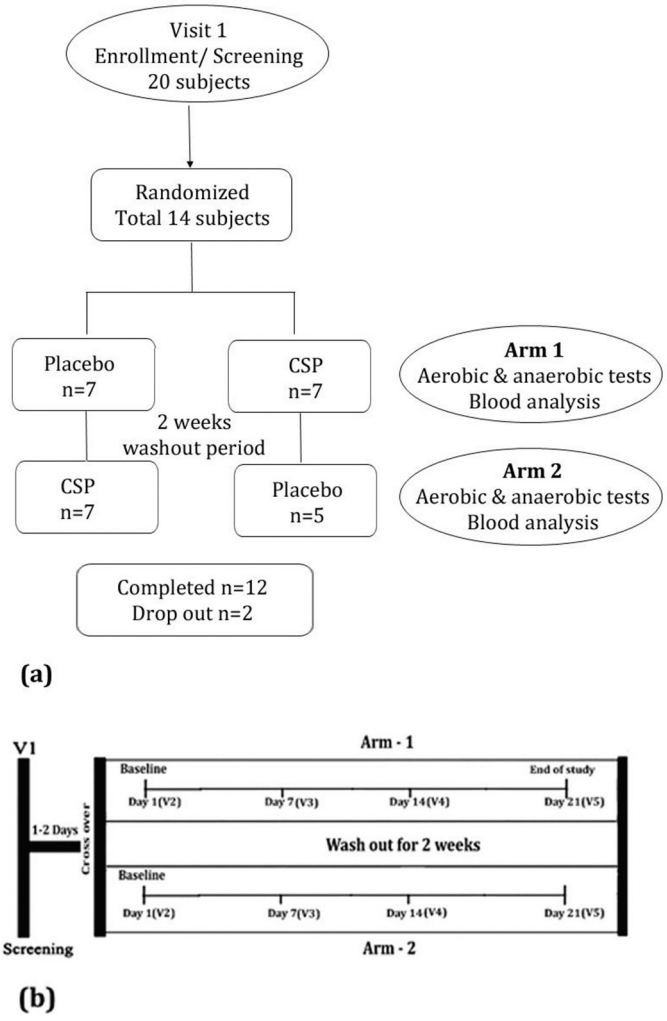

Fig. 1. (a) Consort diagram showing the flow of participants through each phase of the randomized crossover trial; (b) Trial schedule showing the visits, administration of coconut inflorescence sap powder and placebo, and collection of blood samples.

This protocol was repeated on day 7 (visit 3), day 14 (visit 4) and day 21 (visit 5). Efficiency parameters of the tests such as speed, time, and the number of repeats were recorded individually on each visit. All the participants completed the exercise tests within a 90 ± 10 min period. After the 14 day washout period, the treatment regime was reversed and the visits from day 1 to day 21 were repeated using the same protocol as specified in Arm 1 (Fig. 1). Participants were instructed to continue their general aerobic and anaerobic exercise patterns for the duration of the study. All participants consumed a controlled food pattern involving typical south Indian diet composed of breakfast (rice-based with an egg), afternoon lunch (rice with egg or chicken or fish), dinner and evening snacks (wheat-based with fish or chicken) consisting on an average of 10% fat, 10–30% proteins and 40–65% carbohydrates [12]. This dietary control was provided throughout the study period to all candidates (both placebo and CSP condition) to avoid any influence of diet on performance.

2.4. Anaerobic and run testing protocols

A Running-based, Anaerobic Sprint Test (RAST) was conducted as an anaerobic test that consisted of 6 × 35 m maximal sprints interspersed by 10 s passive recovery periods. Each sprint began from a standing start position and the sprint time was recorded. The sprint power was then calculated according to Zagatto et al., as per the equation $\boldsymbol{Power} = (\boldsymbol{Body\ Mass} \times \boldsymbol{Distance}^2)/\boldsymbol{Time}^3$ [13]. Sprint power is a measure of the highest power output and provides information about strength and maximal sprint speed. The higher score indicates the athlete's ability to maintain anaerobic performance.

The 2.4 km run test was performed on a track as described by Haff & Dumke [14]. Subjects were instructed to run as quickly as possible and the time for completion was recorded to the nearest 0.1 s. Peak oxygen consumption, or VO_2 max, was estimated from the speed and body mass according to the equation of George et al., [15]. The equation used to calculate VO_2 max was $\boldsymbol{VO_2\ max}\ (\boldsymbol{mL.kg^{-1}.min^{-1}} = \mathbf{88.02} + (\mathbf{3.716} \times \boldsymbol{gender}) - (\mathbf{0.0753} \times \boldsymbol{body\ weight\ in\ pounds}) - (\mathbf{2.767} \times \boldsymbol{time\ for\ 2.4km\ in\ minutes})$ in which gender = 1 for males and 0 for females VO_2 max is the maximum amount of oxygen in milliliters that is utilized in one minute per kilogram of body weight (mL/kg/min). Those who have a higher VO_2 have a greater aerobic capacity.

2.5. Blood clinical markers

Fasting blood samples and the blood samples before and after the physical tests were collected into heparinized and non-heparinized tubes from the antecubital vein, to analyze fasting blood sugar level, other hematological and biochemical markers. To separate serum, the blood was allowed to clot for 30 min and centrifuged at 1000 ×*g* at 4 °C for 10 min. Plasma was separated from the heparinized tubes by the same centrifugation protocol. Both serum and plasma samples were stored at −20 °C for later analysis. Hematological parameters include hemoglobin (Hb) content, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) levels. Excluding Hb, all other assays were done by assay kits provided by M/s Agappe Diagnostics Pvt. Ltd., Bangalore, India. All assays were performed by a certified medical professional in quadruplicate and the average value was reported.

Serum lactate dehydrogenase (LDH) activity was also determined by a colorimetric assay at 550 nm, using kit method (Cat. No: ab102526; M/s Abcam, Cambridge, USA) [16]. Antioxidant status was measured by estimating serum level activities of superoxide dismutase (SOD) [17], Catalase [18] and glutathione peroxidase (GPx) [19].

Inter- and intra-assay coefficients of variation (%CV) in blood marker analysis was performed as follows. Intra-assay %CV was calculated as the ratio of the pooled standard deviation from all samples (each was analyzed in quadruplicate) to the overall mean, multiplied by 100. Inter-assay %CV refers to assay-to-assay consistency which was calculated as the ratio of the pooled standard deviation to the overall mean of all duplicated samples, multiplied by 100. Mean intra-assay CV for the blood markers was found to be 6.01% and the mean inter-assay CV was 2.96%.

2.6. Statistics

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS–25) for both placebo and CSP condition. A 2 -way repeated measures Analysis of Variance (ANOVA) was performed to analyze the data. A 2 × 4 Repeated measures ANOVA was also carried out to find out the progress in mean power in condition *vs* time. Differences were considered significant at $p < 0.05$. Reported values are arithmetic means with standard deviation (SD) or standard error of the mean (SEM) as indicated.

3. Results

3.1. Characteristics of CSP

CSP prepared by a proprietary process at controlled conditions of temperature and pressure to preserve the micronutrients in coconut inflorescence sap was employed as the test substance in the present study (CSP; registered as COCOZEN®). It was stable and does not undergo fermentation under ambient conditions (25 ± 2 °C, relative humidity 60 ± 5°). Physicochemical characteristics and various micronutrients in the standardized CSP are given in Tables 1 & 2 respectively. It was found that CSP contains 2 and 5 fold higher levels of vitamins (B and C), 20 and 15 fold electrolytes (Na and K) and around 3, 11, 40 and 16 fold minerals respectively (Ca, P, Fe, and Zn) than coconut water. It also contains 1% Nitrate. The micro and macronutrients composition of CSP and Placebo per serving are provided in Table 3.

3.2. Study subjects

Fourteen apparently healthy males (age 20 ± 1.5 years; BMI 22.99 ± 0.79) were enrolled and 12 subjects completed the study (Fig. 1). Two subjects voluntarily withdrew from the study.

Baseline anthropometric measurements and safety parameters were in the normal range (Systolic BP were 123.07 ± 7.34 in placebo and 124.60 ± 5.44 mmHg in CSP group; Diastolic BP were 81.20 ± 6.74 in placebo and 78.53 ± 6.79 mmHg in CSP; Heart rates were 74.07 ± 1.22 in placebo and 69.20 ± 2.21/min in CSP group). Safety parameters were in the normal range: AST was 26.13 ± 7.74 U/L in placebo and 36.20 ± 7.74 U/L in CSP group ; ALT was 29.60 ± 11.41 U/L in placebo and 34.07 ± 10.80 U/L in CSP; ALP in placebo 72.40 ± 18.01 U/L and in CSP 83.27 ± 25.57 U/L respectively). All these parameters remain normal at the end of study which is presented in Table 4. No significant body

Table 1
Physicochemical characteristics of Coconut inflorescence sap powder.

Sl. No.	Parameters	Results
1	Colour and appearance	White free flowing granular powder
2	Taste	Sweet
3	Bulk density	0.2–0.5 g/mL
4	Moisture[a]	4.8%
5	Water activity[a]	0.25
6	Ash content[a]	0.95%
7	Solubility	Soluble in water
8	Protein[a]	1.5%
9	Fat[a]	1%
10	Carbohydrate[a]	78%

[a] Official Methods of Analysis of AOAC International, 19th Ed., [9]; Method numbers: Moisture - 43:1(986.21); Water activity - 42:1–2(978.18); Ash content - 43:2 (941.12); Protein - 04:33–36 (2001.11); Fat −04: 40–42 (2003.05); Carbohydrate - 50:18(986.25) & Jose et al., [5].

Table 2
Micro nutrient comparison of Coconut inflorescence sap powder with Coconut water[a].

Nutrient	CSP (100 g)	Coconut water (100 g)
Vitamin C	48.91 mg	9.9 mg
Total Vitamin B	2.40 mg	–
Calcium	23.60 mg	7 mg
Iron	1.2 mg	0.03 mg
Phosphorus	58 mg	5 mg
Zinc	0.32 mg	0.02 mg
Sodium	536 mg	26 mg
Potassium	2614 mg	165 mg
Choline	3.32 mg	–

[a] Nutrient composition of coconut water was taken from US Department of Agriculture Official Website Survey (FNDDS) FDC ID: 1100594 Food Code:42403010. CSP analysis were done as per the methods of Sami et al., [10] & Hussain et al. [11],

Table 3
Nutrient composition of Coconut inflorescence sap powder and Placebo per serving.

Nutrient	CSP (3 g)	Placebo (3 g)
Macronutrients		
Carbohydrates[a]	2340 mg	0.356 mg
Protein[a]	45 mg	0.01 mg
Fat[a]	3 mg	0 mg
Micronutrients		
Vitamin C	1.460 mg	0 mg
Vitamin B	0.087 mg	0 mg
Calcium	0.07 mg	0 mg
Iron	0.03 mg	0 mg
Phosphorus	1.740 mg	0 mg
Zinc	0.010 mg	0 mg
Manganese	0.003 mg	0 mg
Sodium	16.080 mg	0 mg
Potassium	78.420 mg	0.02 mg
Choline	0.100 mg	0 mg
Nitrate	30 mg	0 mg

[a] Official Methods of Analysis of AOAC International, 18th Ed., [9]; Method numbers: Moisture - 43:1(986.21); Water activity - 42:1–2(978.18); Ash content - 43:2 (941.12); Protein - 04:33–36 (2001.11); Fat −04: 40–42 (2003.05); Carbohydrate - 50:18(986.25) Micronutrients are estimated as per the method by Sami et al., 2014 & Hussain et al., 2011. Nutrient composition of Aspartame was taken from US Department of Agriculture Official Website Survey (FNDDS) FDC ID: 1103946 Food Code:9120101.

weight change was noted during the study period. Both placebo and CSP treated condition showed no significant effect ($p > 0.05$) in AST, ALT and ALP levels.

3.3. Performance testing

The results of RAST showed an increase in mean power for both the placebo ($p < 0.05$) and CSP supplemented conditions ($p < 0.05$) (Fig. 2). In addition, CSP supplementation was found to offer a steady and progressive improvement in mean power over 21 days, as compared to the placebo (Fig. 2). Further, condition × time interaction analysis using 2 × 4 repeated measures ANOVA also indicated significant ($p < 0.05$) increase among CSP subjects (Fig. 2). Condition × time analysis of peak power also revealed a significant increase ($p < 0.05$) under CSP condition (46.59%), as compared to the placebo (4.06%) (Fig. 3).

In the 2.4 km endurance run test to estimate peak oxygen consumption (VO_2 max), condition × time analysis showed that CSP significantly increased VO_2 max (5.31%; $p < 0.05$), compared to the baseline values (Pre: 59.38 ± 2.15 mL/kg/min; Post: 62.56 ± 0.52 mL/kg/min); but no differences were noted under the placebo condition (Pre: 59.38 ± 2.28 mL/kg/min; Post: 61.07 ± 0.69 mL/kg/min) (Fig. 4).

Table 4
Anthropometric characteristics and clinical markers of study subjects (n = 12) All data here (Initial and end of study).

Parameters	Time point	Placebo	CSP
Age (years)	Pre	20.00 ± 1.50	20.00 ± 1.50
	Post	20.00 ± 1.50	20.00 ± 1.50
Height (cm)	Pre	175.33 ± 6.90	175.33 ± 6.90
	Post	175.33 ± 6.90	175.33 ± 6.90
Weight (kg)	Pre	70.70 ± 4.39	70.70 ± 4.39
	Post	70.70 ± 4.39	70.70 ± 4.39
BMI (kg/m^2)	Pre	22.99 ± 0 0.79	22.99 ± 0 0.79
	Post	22.99 ± 0 0.79	22.99 ± 0 0.79
Systolic BP (mmHg)	Pre	123.07 ± 7.34	124.60 ± 5.44
	Post	122.02 ± 5.30	126.67 ± 4.05
Diastolic BP (mmHg)	Pre	81.20 ± 6.74	78.53 ± 6.79
	Post	79.60 ± 4.28	84.53 ± 3.74
Heart rate (per min)	Pre	74.07 ± 1.22	69.20 ± 2.21
	Post	70.51 ± 2.69	73.40 ± 2.96
AST (U/L)	Pre	26.13 ± 7.74	36.20 ± 7.74
	Post	27.57 ± 3.70	28.93 ± 6.24
ALT (U/L)	Pre	29.60 ± 11.41	34.07 ± 10.80
	Post	28.74 ± 9.40	32.67 ± 10.46
ALP (U/L)	Pre	72.40 ± 18.01	83.27 ± 25.57
	Post	71.51 ± 17.80	72.07 ± 18.99
FBS (mg/dL)	Pre	92.64 ± 4.57	95.12 ± 3.46
	Post	93.39 ± 5.63	94.65 ± 5.71
SOD (U/mg)	Pre	6.43 ± 0.64	6.53 ± 0.32
	Post	4.20 ± 0.20	8.20 ± 0.41*
Catalase activity	Pre	40.30 ± 2.01	38.66 ± 2.3
(U/mg)	Post	35.96 ± 1.79	45.47 ± 2.26*
GPx (U/mg)	Pre	197.50 ± 9.87	192.90 ± 9.59
	Post	179.90 ± 9.0	255.90 ± 12.75*
Lactate level	Pre	9.14 ± 0.36	9.34 ± 0.35
(Mm/dL)	Post	8.87 ± 0.40	7.96 ± 0.47*

Values are given as mean ± SD. *$p < 0.05$ indicate post significantly differ with pre treatment. The samples were collected 30 min before exercise and immediately after completeion of exercise performance. CSP-Coconut sap powder; BMI-Body Mass Index; AST- Aspartate amino transaminase; ALT-Alanine amino transaminase; ALP-Alkaline phosphatase; FBS- Fasting blood sugar; SOD-Superoxide dismutase; GPx-Glutathione peroxidase.

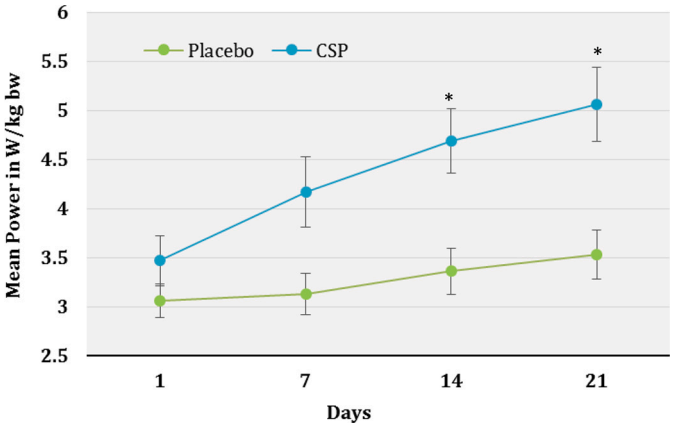

Fig. 2. Progressive trend line showing Mean power in participants. Mean ± SD power in healthy men ($n = 12$) after daily intake of placebo or Coconut inflorescence sap powder supplementation, from days 1 to 21. Statistical significance was analyzed using 2 × 4 Repeated measures ANOVA with time × condition comparison (SPSS-25). The placebo significantly differ with Coconut inflorescence sap powder at $p < 0.05$.

3.4. Blood clinical markers

Compared to baseline, the antioxidant enzyme activities of SOD, CAT, and GPx were lower ($p < 0.05$) in the placebo condition (Table 4). CSP, however, increased SOD (Pre: 6.53 ± 0.32, Post: 8.2 ± 0.41), CAT (Pre: 38.66 ± 2.3, Post: 45.47 ± 2.26 and GPx activities (Pre: 192.9 ±

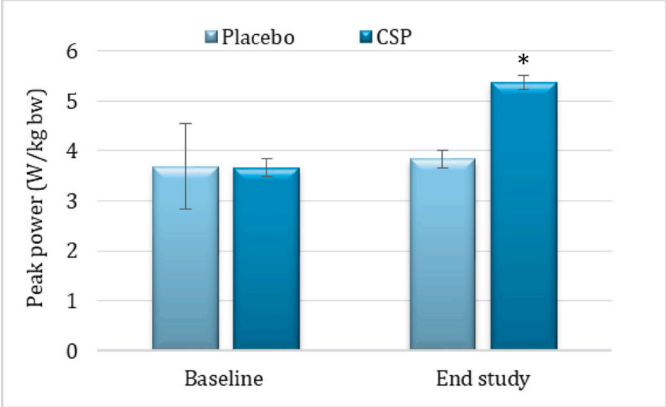

Fig. 3. Peak Power (in W/kg bw) of subjects from RAST. Time × condition interaction was analyzed using 2 × 2 Repeated Measures ANOVA. Values are expressed as Mean ± SD. '*' represent significant difference at the end of study compared to baseline, $p < 0.05$.

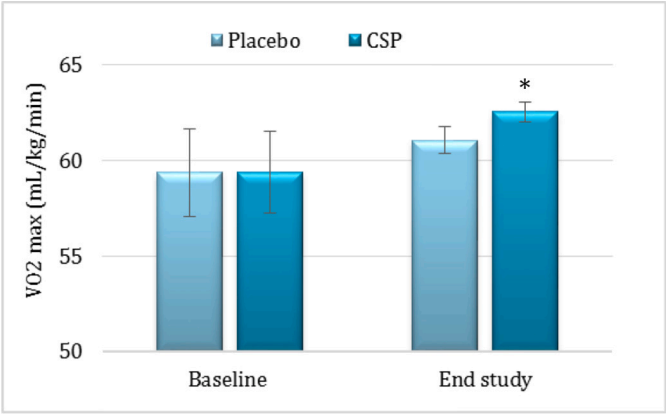

Fig. 4. VO_2 max of subjects from 2.4 km running test. A within-condition comparison was done by 2 × 2 Repeated measures ANOVA. Values are expressed as Mean ± SD. '*' represent significant difference at the end of study compared to baseline, $p < 0.05$.

9.6, Post: 255.9 ± 12.75) at the end of the study period compared to the baseline (all, $p < 0.05$) Serum lactate concentration also increased ($p < 0.05$) in both conditions during heavy exercise. CSP, however, attenuated the rise in lactate concentration ($p < 0.05$; Pre: 9.34 ± 0.35, Post: 7.96 ± 0.47). In conclusion, SOD, CAT, GPx and lactate level were significantly altered in CSP condition × time interaction.

4. Discussion

This is the first study to investigate the ergogenic effect of coconut inflorescence sap using a novel powder formulation (CSP) suitable for storage and supplementation as sachets or powdered drink. The current findings demonstrated that 21-days of CSP supplementation (3 g/day) increases mean and peak anaerobic power and aerobic performance with a concomitant increase in endogenous antioxidant enzyme activity. Thus, the findings of the study suggest CSP may be a novel natural intervention agent rich in micro- and macro-nutrients to improve exercise performance in young, apparently healthy adults.

The oyster–white translucent coconut inflorescence sap is a traditional health drink in many Asian countries. It is reported to contain various vitamins (B1, B3, B5, B7, B8, B9, C), and minerals (Na, K, Ca, Mg, P, Fe, Cu, Zn) in addition to nutrients like biotin, choline, and polyphenols [20]. Rapid fermentation of the sap to alcohol, however, has greatly limited its accessibility as a performance and/or health-enhancing drink. The most popular form of coconut inflorescence sap is coconut sugar, which is a natural sweetener with relatively low glycaemic index (GI = 52) *versus* the table sugar with a high glycemic index of 65 to 70 [5]. Glycemic Index is a relative ranking of carbohydrates in foods in terms of their ability to affect blood glucose levels. Carbohydrates with GI 70 was considered as high, 56–70 as medium and below 55 are considered as low, based on the reface of glucose which has been assigned a GI of 100 [21]. However, the conventional high-temperature evaporation process of preparation of coconut sugar has been shown to destroy the micronutrients in coconut sugar, despite its use as a nutrient-rich natural sweetener. Recently, we had reported the GI of CSP as 52.4 [22].

Our findings demonstrate that CSP has greater concentrations of vitamins (C and B), electrolytes (Na and K), and minerals (Fe, Zn, Ca, P, Mg) than the commonly available coconut water (Table 2). But, nutritional analysis of CSP in comparison with coconut sugar and table sugar indicates that the carbohydrate content and energy of CSP are similar. Carbohydrate-enriched electrolyte drinks have been reported to improve performance [23,24]. Moreover, vitamins and minerals are key regulators of health and function, including work performance [25]. Micronutrients enable the use of macronutrients for all physiologic processes [26]. Vitamins A, C, E, and the minerals selenium and zinc are the non–enzymatic line of defense against reactive oxygen and nitrogen species which are generated in excess during exercise [27]. Zinc is a cofactor for many enzymes and has a role in tissue repair [28]. Similarly, Iron has the principal function in hemoglobin whose deficiency is associated with the reduction in oxygen-carrying capacity and decreased exercise performance [28]. Deficiencies of vitamin B and C were reported to impair exercise performance and lead to a significant decrease in VO_2 max and the ventilatory threshold [29]. Choline is involved in the formation of acetylcholine, a neurotransmitter whose depletion in the nervous system has been correlated to fatigue [30]. Plasma levels of sodium and potassium are extremely important for performance since fluid-electrolyte balance is very important in muscle contractility and transport glucose into the muscle cell [31,32]. Potassium is the primary intracellular electrolyte mostly present in muscle fibers along with glycogen.

In the present study, supplementation of CSP at 3 g/day for 21 days did not produce any adverse events and no significant change in the blood clinical markers related to liver function indicating its safety and tolerance at the tested level. It did not produce any change in the fasting blood sugar, though rich in carbohydrates (Table 4). Further evaluation of its ergogenic potential was carried out with the help of a set of validated physical tests in a crossover design. Crossover design has the advantages over placebo-controlled design in evaluations such as the efficacy of a sports supplement because individuals act as a control for themselves for better sensitivity [33]. The CSP condition demonstrated a significant improvement in both anaerobic and aerobic performance during the study period. Anaerobic exercise is an intense physical activity of very short duration fuelled by the energy sources within the contracting muscles, largely independent of oxygen [34]. Running based anaerobic sprint test (RAST) is yet another important anaerobic test widely used for the investigation of anaerobic power in terms of Peak and Mean power as the variables for efficacy [13]. Ballmann et al. [35] reported a 1.2 W/kg b. wt. increase in anaerobic power upon *Rhodiola rosea* (Golden Root Extract) supplementation in a short term study using 1.5 g/day dosage. In another study using 'Assault™' (a dietary supplement containing caffeine, branched chain amino acids, creatine, beta-alanine, arginine, vitamin B-6, and vitamin B-12) for four weeks lead to significant improvement in anaerobic peak power (8%) compared to placebo [36]. Thus, our findings with CSP showed similar efficacy for improving anaerobic performance compared to other dietary interventions.

Endurance exercise is regarded as an activity that uses large muscle conditions continuously in a rhythmic nature [37]. The 2.4 km run test has been used to measure endurance in terms of peak oxygen

consumption (VO_2 max) along with reduction in total test time [38]. The ability to utilize a high fraction of VO_2 max for a longer duration is an indication of greater endurance performance [39]. The enhanced VO_2 max observed in CSP condition indicates its capacity to enhance performance. This is in line with previous studies of Ghiasvand et al. who reported 6% improvement in VO_2 max compared to placebo when β-alanine was supplemented at 2 g/day for 6 week [40]. Another review by Pasricha et al. [41] stated that iron supplementation significantly increased the relative VO_2 max [0.82–3.88 mL/(kg.min)]in healthy female subjects. In addition, various studies have substantiated the role of micronutrients in performance and energy [28]. So, the improvement in endurance and anaerobic performance upon CSP condition may be attributed to the significant levels of micronutrients and their bioavailability. The polyphenols presented in CSP may also have some beneficial roles in its activity [42].

CSP was shown to lower oxidative stress associated with exhaustive exercise in the present study. High muscle activity is associated with the generation of reactive oxygen and nitrogen species (ROS and RNS) with a simultaneous elevation in the body's antioxidant defense system including SOD, Catalase and GPx [43]. CSP condition increased GSH, an important antioxidant involved in the detoxification process. SOD is yet another endogenous oxidoreductase involved in the dismutation of superoxide anion into molecular oxygen and hydrogen peroxide; which was also increased in CSP condition. Similarly, GPx, an antioxidant enzyme involved in the detoxification of H_2O_2 with the help of GSH was also found to be elevated to the normal level in CSP condition, suggesting a role of antioxidant cellular protection during exhaustive exercise. The involvement of free radicals in the peroxidation of membrane phospholipids and formation of lipid peroxide or hydrogen peroxide has been reported under conditions of oxidative stress and exercise. Further, lactate dehydrogenase (LDH) is also an important enzyme marker of muscle activity and increases with exhaustive exercise [44,45]. Lactate dehydrogenase (LDH) catalyzes the interconversion of pyruvate and lactate, which are critical fuel metabolites of skeletal muscle particularly during exercise [45]. The present study showed a decrease in LDH activity upon CSP supplementation which may be considered as an indication of reduced muscle damage [46]. This is in line with previous studies [44]. Collectively, these findings suggest that CSP may improve antioxidant systems and lower LDH activity to improve performance.

Despite the fact that the study could implement the design with a successful gathering of information, major limitations include the small sample size and lack of a double-blinded placebo-controlled design. Another limitation is the estimation of VO_2 max using an indirect method. Future studies following randomized, double-blinded placebo-controlled studies employing higher population and instrumental techniques such as indirect calorimetry would be warrented to substantiate the results.

5. Conclusions

In summary, the present study shows the efficacy of CSP to improve physical performance, while demonstrating safety and tolerability. CSP condition improved both anaerobic and endurance exercise performance that was associated with a concomitant increase in plasma antioxidant activity. However, this is a preliminary study which supports follow up investigations to elucidate the efficacy and mechanism (s) for CSP in improving physical performance.

CRediT authorship contribution statement

Ashish Joseph: Conceptualization and Methodology, Formal analysis, Data Curation; **Svenia P. Jose:** Data Curation; **Bintu T. Kalyan:** Conceptualization and Methodology, Formal analysis, Data Curation; **Ratheesh Mohan:** Manuscript drafting; **Renny R. Mammen:** Resources; **Krishnakumar I. M:** Conceptualization, Visualization, Review, Editing & Resource; **Bradley S. Fleenor:** Review and editing.

Funding

No external funding was received for the study.

Declaration of Competing Interest

Authors disclose the conflict of interest. CSP is the patent-pending formulation of coconut inflorescence sap by M/s Akay Natural Ingredients Ltd., Cochin, India; trademarked as COCOZEN®.

Acknowledgments

The authors would like to thank Coconut Development Board, India for the unfermented coconut infloresence sap for manufacturing CSP.

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