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Improved Oxygen Saturation and Performance of Athletes using *Cordyceps militaris*

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ABSTRACT

Background and Objective: Athletes put a lot of energy to compete with their fellow athletes. Here energy and performance difference among the top 10% athletes is very little. Hence athletes will be constantly improving their energy and performance by doing practice and supplementations. Hence, the objective is to evaluate *Cordyceps* for improved athlete performance. **Materials and Methods:** In the present study, 48 athletes of age group 16-35 were divided into four groups of 12 athletes each and supplemented with blind plebaco control, *Cordyceps militaris* active mushroom infusion 1g/day test, 10 g/day way protein control and another group supplemented with *Cordyceps militaris* active mushroom infusion 1 and 10 g/day way protein. All the groups were supplemented for 3 weeks and athletes were tested for oxygen saturation as SpO_2 and fatigue time as the time athlete can run on the treadmill and on track for running 5 km and 200 m distance. Haematological studies were analysed in blood samples collected before and after supplementation studies with treadmill and running tests. **Results:** It was observed that in *Cordyceps militaris* supplemented group oxygen saturation is high (95%) heart rate is stabilized (90±5), running time for 5 km (13.5 min) and 200 m (25 sec) is significantly less than the control. Fatigue on a treadmill is decreased by 2.5 fold compared to control. In *Cordyceps militaris* supplemented subjects, RBC size increased leading to an increase in Hb, HCT, oxygen carrying capacity and oxygen flow and leading to oxygen saturation and reduced fatigue. **Conclusion:** Only *Cordyceps*-supplemented subjects showed better performance with oxygen saturation and less fatigue compared to *Cordyceps* and protein supplemented subjects.

KEYWORDS

Runner athletes, *Cordyceps*, oxygen saturation, performance, endomorphic fungi, endurance

INTRODUCTION

In athletes fatigue and lower performance are common and can affect the peak of their career. After reaching a peak further improvement becomes slow or difficult. However, any improvement after peak saturation will help the athletes to become winners. *Cordyceps*, a genus of fungus, has been used in traditional medicine of China to treat fatigue and respiratory, kidney and heart problems[1]. More recently the benefits of *Cordyceps* for athlete performers have been evaluated[2]. *Cordyceps* was first linked with 1993 world record performance of a Chinese athlete. *Cordyceps sinensis*, a parasite caterpillar was reported

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for performance improvement studies due to cordycepin[1,3]. Due to the easy cultivation process and high cordycepin, *Cordyceps militaris* is grown at a large scale and is used for medicinal applications. As natural exercise promotes[4] *Cordyceps* also promotes blood flow and oxygen utilization and enhances the clearance of lactate[5]. This will allow the athletes to maintain a higher intensity of exercise, while the reduction in oxidation stress and clearance of lactate, leading to delay of fatigue[6-8]. Despite potential benefits, a few studies have found no benefits of *Cordyceps* supplementations[9,10]. There is no conclusive study on the type of material, dosage, additional supplements and outcome measurement methods. Higher doses of dry *Cordyceps* sinensis 4-6 g/day resulted in increased oxygen saturation and lactate clearance[11] but lower doses were not effective[9]. The present study is carried out to understand the effect of low doses of live *Cordyceps militaris* mushroom infusion on the maintenance of oxygen saturation and improvement of running performance or endurance. Simultaneously protein supplementation was also tested for improvement of oxygen saturation and running performance or endurance.

MATERIALS AND METHODS

The research is carried out at Nizam College, Hyderabad, India. The study was carried out within 3 weeks; 5-26 January, 2022. Before testing, all participants signed a written informed consent and underwent a 12 lead electrocardiogram, physical examination, complete blood picture, hematocrit, size of RBC, oxygen saturation on 3 min treadmill run at 150 rpm and time of tiredness on a treadmill and running track (endurance). Two weeks before the commencement of experiments, the above baseline data was collected in two visits on two consecutive days at 10 am. In a randomized, double-blind, pelbaco-controlled design, participants were randomly assigned using random allocation software (RAS 2.0) into one of four treatment groups. Pelbaco control[1], *Cordyceps militaris* infusion supplement group[2], protein supplement group[3], protein and *Cordyceps* infusion supplement group[4]. The study was approved by an institutional ethical committee.

Subjects were provided only 150 mL black tea as control[1], 150 mL black tea with 1g active alive *Cordyceps militaris* mushrooms infusion as test group[2], 150 mL black tea with 10 g way protein was given as protein control[3] and 150 mL black tea with 10 g protein and 1g mushroom infusions were given to 4th group. All infusions were similar in colour and taste. Subjects were assigned with code numbers and test and control infusions were provided as per codes.

Following three-week supplementation period subjects were examined for 12 lead electrocardiograms, physical examination and haematocrit, size of RBC and oxygen saturation on a treadmill run at 150 rpm and time of tiredness on the treadmill and running on a track.

Subjects: A total of 48 young non-obese (BMI<28 kg/M2) subjects were selected for participation in the study. These adults (24 males and 24 females) between the ages of 16-35 years completed the study. All the subjects selected for the study are runners practising running for minimum 1 to 5 years under the guidance of Olympic athlete coach Mr. Bhaskar Reddy. Subjects don't have musculoskeletal injury, cardiovascular diseases, diabetes, allergy to mushrooms and agreed to abstain from alcohol, tobacco and running practice 24 hrs before testing days but otherwise maintain their diet and exercise for the duration of the study.

Ethical consideration: A written consent form was taken from all participants. Approval was sought and obtained from the Research Ethical Committee of Nizam College, Osmania University, Hyderabad, before the commencement of the study.

Materials: *Cordyceps militaris* was isolated earlier in the laboratory (Department of Microbiology, Nizam College) from fruiting bodies collected and strain improved to give more cordycepin production[12]. *Cordyceps militaris* was grown in solid state fermentation and fresh fruiting bodies were used for the present study.

Supplementation: Throughout the 3-week intervention period, participants consumed 150 mL of either only black tea, only 1 g *Cordyceps militaris* infusion prepared in 150 mL black tea, only 10 g whey protein in 150 mL black tea, 1 g *Cordyceps militaris* and 10 g whey protein in 150 mL black tea. In a litre water 10 g tea powder, 25 g sugar and 5 drops of vanilla were added and boiled for 5 min to prepare the black tea. After boiling it was filtered and provided to the control group. In black tea infusion, *Cordyceps militaris* live ground mushroom was added to make 1 g/150 mL. After boiling for 5 min, filtered and provided to *Cordyceps militaris* test group. To the filtered black tea, whey protein was added at a concentration of 10 g/150 mL and provided to the third group. To the infusion prepared with *Cordyceps* in tea, whey protein is added to get 10 g/150 mL and provided to the 4th group.

Procedures

Oxygen saturation and heart rate: Blood oxygen saturation and heart rate of the subjects under study were measured by placing OXY-MED Pulse Oximeter (Medex equip Healthcare Solutions Ltd., India) on pre-cleaned and dried index finger. Before running on a treadmill and on track, a pulse Oximeter was placed on the index fingers of subjects. After recording oxygen saturation and heart rate at zero time (resting time) subjects were allowed to run on a treadmill or running track and oxygen saturation and heart rate were recorded.

Three-minute maximal treadmill test: After placing OXY-MED pulse Oximeter on index finger, subjects were asked to run on a treadmill (True Alpine). Following a two minutes self-placed warm-up at 100 rpm, speed was increased to 150 rpm and the timer was started. Subjects were not given time updates throughout the test, oxygen saturation as SpO_2 and heart rate were measured through a pulse Oximeter. Tiredness is measured when the subject gives up on a treadmill and endurance is measured as time duration of an individual running on a treadmill or track.

Running performance on the track: Four study groups were selected as mentioned in design. Each group consists of 12 athletes (6 males and 6 females) from which 6 athletes (3 males and 3 females) are short distance (200 m) runners (spinsters) and 6 athletes (3 males and 3 females) are long distance (5000 m) runners. After placing OXY-MED pulse Oximeter on index finger, subjects were asked to run on track used for running. During short and long distance running high definition (HD) video was recorded. From video, oxygen saturation (SpO_2), heart rate and endurance were noted.

Hematological analysis

Collection of blood samples: The blood samples were drawn from volunteer athletes. The blood was collected from the left arm vein for each subject. The 3 mL blood was drawn from each subject. The blood was taken into centrifuge tubes containing heparin.

Hemoglobin determination: Hemoglobin was estimated by Drabkin's and Sahli methods[13].

Red blood cell count: To enumerate Red Blood Cells (RBCs), blood samples anticoagulated with heparin were utilized. The blood was carefully drawn into the RBC diluting pipette, precisely reaching the 0.5 mark, employing a gentle suction method on the mouthpiece. Subsequently, the lip of the pipette was meticulously cleaned to eliminate any residual blood before immersing it in the diluting fluid (toission solution). The diluting fluid was aspirated up to the 101 mark on the bulb. To ensure a consistent dispersion of blood cells within the pipette, the tube was rotated horizontally. The RBC count was determined using the formula[14]:

$$\text{RBC (million / mm)} = \frac{\text{Cell counted}}{5 \times 10 \times 200}$$

Packed Cell Volume (PCV) and haematocrit: The determination of packed cell volume involved employing heparinized blood in standard capillary tubes (75×1 mm). The tubes were filled to approximately 1 cm from the end, after which the vacant ends were carefully sealed by exposure to flame, ensuring that the blood was not subjected to heat. Subsequently, the capillary tubes were securely positioned within the hematocrit centrifuge machine and centrifugation was conducted at 13000 rpm for a duration of 5 min.

Using a hematocrit reader (Thermo scientific, Hyderabad), measured the length of the column of the packed red cells and divided it by the length of the whole column of blood (cells and plasma), multiplied this number by 100%.

Measurement of RBC size: Blood samples were uniformly spread onto meticulously cleaned slides. The laser diffraction technique employed adheres to the Babinet principle[15], generating a Fraunhofer diffraction pattern on the observer's retina. The computational approach for determining the average size of diffracting particles relies on measuring the diffraction angle. This involves adjusting the sample distance from the central hole to ensure that the diffraction ring on the observer's retina attains the appropriate diameter on the ergometer. Typically used for sizing spherical particles, this method is applied to ascertain the size of red blood cells on prepared slides, as previously documented[16].

Measurement of clotting time (CT)

Capillary tube method (Wright's method): Under sterile precautions made a sufficiently deep prick in the fingertip. Noted the time when bleeding started (stopwatch was started). The blood drop at the fingertip is transferred into a capillary tube. Tilt the capillary tube downward to facilitate filling through capillary action. After approximately 2 min, begin snapping off small sections of the tube at 15 sec intervals, carefully observing whether a fibrin thread forms between the snapped ends each time. Record the time when the first appearance of the fibrin thread is observed.

Blotting time (BT): Duke's technique involved sterilizing the fingertip with spirit and allowing it to dry. A sterile lancet was used to make a sufficiently deep prick, enabling blood to flow freely without additional pressure. The start time, indicated by the initiation of bleeding, was noted using a stopwatch. The blood was gently absorbed by touching the fingertip with a filter paper. This process was repeated every 15 sec, with a fresh portion of filter paper each time, until bleeding ceased. The stopwatch was then stopped and it was observed that the blood stains on the filter paper gradually diminished and eventually disappeared as bleeding came to a halt.

Measurement of lactate: Right after collection, 200 µL of blood was promptly transferred into each of the two test tubes, each containing 2 mL of cold perchloric acid. The perchloric acid extracts were then frozen at -80°C and later analyzed for lactate. The determination of whole blood lactate was carried out through a spectrophotometric modification of the enzymatic technique initially outlined by Powers *et al*.[17], utilizing a commercially available kit.

Statistical analysis: Experiments were carried out for 3 weeks, baseline data was collected two times and test and control experimental observations of 4 groups data was collected 3 consecutive days of each week. Separate and mixed factorial (treatment X time) ANOVA with Excel of Microsoft Office was carried out; comparisons were used to analyse differences in primary variables (SpO_2, Hb, hematocrit, RBC size, endurance etc.). Ninety-five percent confidence intervals ($p<0.05$) were considered as statistically significant. Results are shown in Table 1, along with standard deviation.

RESULTS

Oxygen saturation and heart rate: Oxygen saturation (SpO_2) was decreased sharply from 100% to 60-75% in control and protein supplemented groups within a minute. Whereas *Cordyceps militaris* infusion

Table 1: Effect of *Cordyceps militaris* supplementation on hematological and performance of athletes along with standard deviation

Group test	Black tea control (group 1)	*C. militaris* test 1+1 g/day *C. militaris* infusion (group 2)	Protein control 1+10 g/day way protein (group 3)	*C. militaris*+protein test 2+10 g/day way protein (group 4)
Hematological observations				
RBC count	5.8±1 mCL	5.83±0.5 mCL	5.84±1 mCL	5.84±0.8 mCL
RBC size	6.5±1 µ	7.8±0.8 µ	6.6±0.6 µ	7.2±1 µ
Hemoglobin content	15±0.25 g dL^{-1}	16.2±0.5 g dL^{-1}	15.2±0.7 g dL^{-1}	16±1 g dL^{-1}
Hematocrit	38±0.9%	45±0.55%	39±0.8%	42±0.75%
Lactate	4.5±0.68 mM	0.1±0.78 mM	5±0.66 mM	0.5±0.55 mM
Clotting time	3±1 min	4±1.4 min	3 ±0.77 min	4±0.89 min
Blotting time	2.5±0.8 min	3.2±0.5 min	2.7±0.68 min	3.1±0.55 min
Treadmill and running observations				
Minimum oxygen saturation SpO_2	72±1%	95 ±1.5%	68±1.8%	93±1.2%
Heart rate (beats per minute)	130±15	90±5	140±10	95±7
Treadmill test time	2±3 min	5±1 min	2.2±0.5 min	4±1.1 min
5 km running time	18 ±1.4 min	13.5 ±1 min	17±1.2 min	14.7±1.8 min
200 m running time	40±1 sec	25±2 sec	35 ±1 sec	28 ±0.5 sec

supplemented subjects showed 95% till the end of the 5 min extended treadmill test and on track. The group supplemented protein along with *Cordyceps militaris* infusion showed less saturation (93%) than only *C. militaris*. Heart rate is normally increased with running and exercise as witnessed by normal and protein controls. However, heart rate is stabilized in *C. militaris* infusion supplemented subjects (Table 1).

Maximal treadmill test: The maximum time one can perform a treadmill test was taken as endurance. At 150 rpm on the treadmill control group subjects showed SpO_2 less than 70%, 130±15 heart rate and tiredness in 2 min. Protein supplemented group also showed similar observations to the control. Whereas, *C. militaris* infusion supplemented group subjects showed higher oxygen saturation (SpO_2, 95%), stabilized heart rate (90±5) and were able to perform running on a treadmill for 5 min. Protein supplements along with *C. militaris* also increased the SpO_2 and endurance but less than the *C. militaris* alone.

The athletes with more years of practice require more days of *Cordyceps militaris* infusion supplementation for SpO_2 saturation and endurance. Whereas, fresh or less trained athletes achieved 95% SpO_2 and got high endurance early of *Cordyceps militaris* infusion supplementation.

Running performance on the track: From each group running performance was evaluated for long distance and short distance by different dedicated subjects. There is a significant reduction of time for long distances 5 km (13.5 min) and short distances 200 m (25 sec) runners of *Cordyceps militaris* infusion supplemented subjects compared to normal control (18 min/40 sec) and protein control (17.5 min/35 sec). There is a maximum time reduction in short-distance runners compared to long-distance runners. In *Cordyceps militaris* infusion and protein supplemented group time reduction for running is observed but less than only *Cordyceps militaris* infusion supplemented group.

Hematological observations: Total number of RBC was maintained almost the same in all tests and controls. The RBC size 7.8 µ, Hb 16.2, HCT 45% were increased in *Cordyceps militaris* infusion supplemented subjects. *Cordyceps militaris* infusion and protein supplemented group also showed a significant increase in RBC size 7 µ, Hb 16, HCT 42% compared to control RBC size 6.5 µ, Hb 15, HCT 40%. The RBC number is constant and an increase in Hb and HCT is correlated with increased RBC size. Lactate content is reduced to almost negligible. *Cordyceps militaris* infusion supplemented subjects (0.1 mM) and *Cordyceps militaris* infusion and protein supplemented subjects (0.5 mM) compared to control (4.5 mM) and only protein supplement group (5 mM).

Clotting time (4 min) and blotting times (3.2 min) were slightly increased in *Cordyceps militaris* infusion supplemented subjects compared to control (CT 3 min/BT 2.5 min). But the increased values are within the range of normal.

DISCUSSION

In the present study, supplementation of *Cordyceps* infusion was found to improve the athlete's performance. *Cordyceps militaris*, a member of Ascomycotic mushroom holds commercial value due to its elevated levels of bioactive substances and its feasibility for artificial cultivation[12]. Cordycepin (3'-deoxyadenosine) is particularly linked to the pharmacological effects of *C. militaris*. *Cordyceps* is being used in various medicinal applications. There are reports of its uses for athletes to improve their performance[2,17,18]. *Cordyceps* (3.15 g/day for 5 weeks) was compared with a placebo to evaluate its effects on physical performance and was found to be effective[19]. The results of the present study demonstrate that a 3-week supplement of 1g/day live *Cordyceps militaris* mushroom infusion has significantly improved oxygen saturation, running performance and reduced fatigue[20]. Increased RBC size by increasing RBC volume promotes oxygen flow and saturation and subsequently helps in the improvement of endurance in athletes[21]. The present study was in agreement with this. In previous studies, 4 g/day dosage of dry *Cordyceps* blend was given and found to be effective after 3 weeks and above time[2]. In earlier studies dried fruiting bodies were used and in the present study live *Cordyceps militaris* mushroom fruiting body infusion is found to be effective at 1 g/day dosage.

Improved aerobic performance through *Cordyceps militaris* supplementation can be ascribed to heightened blood flow and enhanced oxygen utilization, achieved by increasing vasodilation and improving metabolic efficiency[1,22]. As well as increased oxygen exchange by RBC size enlargement as found in this study and anaerobic performance can be achieved by lactate clearance. Enhanced oxygen delivery increases exercise tolerance and delays the onset of fatigue. In the study of Hirsch *et al.*[2] 4 g/day dry *Cordyceps militaris* supplementation does not improve performance but a low dosage of 1-2 g/day for a long duration. After 3 weeks onwards significant increase in oxygen intake was observed. Only way protein supplementation or along with *Cordyceps* did not improve any performance parameters as reported by Jonvik *et al.*[22] and the same was observed in this study.

In rats, *Cordyceps sinensis* exhibited an upregulation of metabolic regulations in skeletal muscles, facilitated angiogenesis and enhanced glucose uptake, thereby improving endurance capacity and delaying fatigue[4]. Additionally, mitochondrial biogenesis increased in the rat model, enhancing fatty acid oxidation and glucose turnover, leading to increased ATP availability and improved performance. *Cordyceps* has also demonstrated antioxidant properties, potentially reducing exercise-induced oxidative stress[5] and enhancing the oxygen availability to endure high-intensity exercise. Athletes who received *Cordyceps militaris* infusion showed a significant improvement in red blood cell size, contributing to enhanced oxygen availability and improved performance. With increased oxygen availability more metabolic activity will be taking place to produce more energy. More energy will lend to improved performance, as found in the present study. Earlier studies also reported oxygen enrichment and clearance of lactase with *Cordyceps* supplementation[11] on running performance by Smith *et al.*[23] and on cyclists by Earnest *et al.*[10] had no effect. But all of the above studies used *Cordyceps* in dry form hence the present study used live *Cordyceps militaris* mushroom grinded and boiled to prepare infusion. *Cordyceps* infusions should be tested in larger populations for improved performance as well as possible side effects in long usage.

Thus with the study of an increased sample number, Cordiceps infusion may be recommended as an energy drink for athletes.

CONCLUSION

Live *Cordyceps militaris* mushroom showed improved performance at 1g/day whereas the same dose of dry blend does not change the performance. This may be due to loss of bioactivity of active components like cordycepin, cyclic AMP etc. in *Cordyceps* due to drying. Only Cordiceps supplemented athletes showed improved performance with oxygen saturation and less fatigue

SIGNIFICANCE STATEMENT

Cordiceps is known for its medicinal values but experimental supporting studies are limited. The present study evaluated the performance of athletes with *Cordyceps* infusion supplementation. Control and protein-supplemented studies were also carried out only *Cordyceps*-supplemented athletes showed improved performance with oxygen saturation and less fatigue. Alkaloids and compounds of *Cordyceps* infusion are enough for improved athlete's performance. Hence fresh *Cordyceps* infusion only should be supplemented for athletes. For improved performance of athletes increased oxygen-carrying capacity and early clearance of lactic acid are required. An increase in erythrocyte size leading to increased oxygen saturation and transportation and early clearance of lactic acid was observed with *Cordyceps* infusion supplementation.

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