Title

A Randomized, Double Blind, Placebo Controlled Study to Evaluate the Efficacy and Safety of Cordyceps Capsules (Food Supplement) as an Add-On Therapy in Patients with Mild to Moderate COVID-19 Infection: from bench to bedside

Background

COVID-19 infection became a global public health concern with limited therapeutic options to treat this condition. Treatment interventions which are effective, safe and tolerable are urgently needed for COVID-19 infection. Due to potential anti-inflammatory, antiviral and lung effects and well characterised safety profile, Cordyceps as was hypothesised to show effectiveness in COVID-19 infection an add on therapy and therefore this double-blind, randomized, placebo-controlled, proof of concept study was planned to evaluate efficacy and safety effect of Cordyceps as a add on therapy in treatment of mild (symptomatic) to moderate SARS-CoV-2 infection. Numerous repurposed and investigational drugs such as remdesivir, chloroquine, hydroxychloroquine, ritonavir, lopinavir and interferon-beta are currently being studied for COVID-19 treatment but their side effects limits their usage among the infected patients. Moreover, COVID-19 vaccine hesitancy is increasing worldwide owing to the fear of side effects. Under such condition, a natural product 'Cordyceps immune booster' with minimal side effects can be a useful. Cordycepin, a pure compound and Cordycepin powder derived from ***Cordyceps militaris*** was tested for their anti-viral properties in ***In Vitro*** assay to detect inhibition of SARS-Cov-19 virus in vero cells Based on our results, it was observed that Cordycepin at 6pM Concentration inhibits 70% of the virus in the assay procedure. The primary objective of the study was **t**o evaluate the efficacy of Cordyceps capsules as an add-on therapy to standard treatment for the treatment of SARS-CoV-2 infection. The s**econdary objectives were t**o evaluate the safety of Cordyceps capsules as an add-on therapy to standard treatment in patients with mild (symptomatic) to moderate SARS-CoV-2 infection and to evaluate the immune modulatory effect of Cordyceps capsules as an add-on therapy to standard treatment in patient with mild (symptomatic) to moderate SARS-CoV-2 infection.

Methods

This was a randomized, double blind, placebo-controlled study of Cordyceps capsules (food supplement) as an add-on therapy in patients with COVID-19 infection. After obtaining informed consent, patients who met all of the inclusion criteria and none of the exclusion criteria was randomized to one of the two groups and received either Cordyceps capsules as an add on therapy to standard treatment protocol or placebo plus standard treatment protocol for the treatment of patients with mild (symptomatic) or moderate SARS-CoV-2 infection. Individual patients' participation was for 30 days.

- **Arm 1 (n=40):** Cordyceps capsules plus standard treatment.
- **Arm 2 (n=40):** Placebo plus standard treatment.

All visits were done in ambulatory setting for both mild and moderate category patients unless the patients with moderate infection was required to hospitalized as per the PI discretion. For hospitalized patients, if the patient was discharged before Day 15 then the patient had to come for Day 16 visit. Day 30 visit was mostly done telephonically. During hospitalization period (at all the other days than that mentioned for trial related investigations), vitals and physical examination, compliance, disease evaluation was done as per the hospital policy. After taking informed consent, eligible patients were randomized to one of the two groups to receive either Cordyceps capsules as an add on therapy to standard treatment protocol or placebo plus standard treatment protocol on Day 1.

Demographics and medical history were taken as a part of screening. Vitals were recorded on all days for hospitalised patients and on Day 1, Day 5, Day 10 and Day 16 for ambulatory visit patients. Complete physical examination was done at screening. At subsequent days, abbreviated examination was done. Height was measured at screening only and weight was

measured at all visit days. X-ray chest and ECG was done on screening visit and on Day 16. RT-PCR test was done on screening if required and on Day 10. Haematology, biochemistry and urinalysis testing were performed on screening, Day 5, and Day 16. Biomarker analysis was performed at screening, Day 5 and Day 10 and includes IL-1, IL-6, MCP-1, IP-10, Ferritin, D-dimer, CRP and induced nitric oxide synthase (iNOS). COVID IgG antibodies testing were done on Day 16. Arterial blood gas analysis (pH, PaO2, PaCo2, HCO3 and SaO2) were performed at screening, Day 5 and Day 10 for moderate category patients. Standard treatment protocol as per recent Clinical Management Protocol for Covid-19, given by Government of India was implemented. Patients received treatment depending on the clinical condition of the patient. Cordyceps 500 mg capsule or matching placebo was administered three times a day after food (e.g., breakfast, lunch and dinner). Cordyceps capsules or placebo were administered at approximately the same time each day as an add-on to the standard therapy. If patient get discharged earlier, he/she was to be taken cordyceps/placebo capsules at home. The total dose of Cordyceps was 1.5 gm per day for 15 days. Cordyceps 500 mg capsule or matching placebo was administered three times a day after food (e.g. breakfast, lunch and dinner). Cordyceps capsules or placebo was administered at approximately the same time each day as an add-on to the standard therapy. If patient get discharged earlier, he/she was to be taken cordyceps/placebo capsules at home.

Results

A total 65 patients were enrolled in the study on Day 1 after confirming eligibility; 33 patients were enrolled in Cordyceps group, and 32 in the placebo group. Out of these, 07 patients (3 in Cordyceps group and 4 in placebo group) did not take any medication and deemed discontinued from the study. Total 58 patients were considered evaluable for the analysis; 30 patients in Cordyceps group and 28 in Placebo group. Out of 30 patients in the Cordyceps group; 27 (90.0%) were of mild category and 3 (10.0%) were of moderate category. Out of 28 patients in the Placebo group; 23 (82.1%) were of mild category and 5 (17.9%) were of moderate category.

Demographics:

Mean age of all the patients enrolled in the study was 42.34 ± 13.61 years. Mean age of patients receiving Cordyceps capsules was 42.55 ± 14.71 years and that of patients receiving Placebo was 42.12 ± 12.59 years. There was male preponderance; 42 (64.61%) were male and 23 (35.38%) were female.

Vitals and physical examination:

Physical examination of patients was normal at all visits. There was no significant change in vital parameters at Day 5, Day 10, and Day 16 as compared to Day 1 visit in patients receiving Cordyceps capsules except in systolic BP and Pulse rate. However, the changes in systolic BP and Pulse rate are not clinically significant.

Chest X-ray:

On Day 1, majority of the patients had normal chest X-ray; 53.3% in Cordyceps group and 64.3% in placebo group. Other important findings were bilateral lower zone infiltrates and ground glass opacities in bilateral lower zones. On Day 16, all patients had normal chest X-ray.

Recovery of patients

Proportionately higher number of patients recovered in Cordyceps group 18 (60%) as compared to Placebo group 15 (53.6%) on Day 5, though the difference was not statistically significant (P value>0.05). Improvement was mainly seen in mild patients.

On Day 10, similar proportion of patients recovered in Cordyceps group 25 (83.3%) and Placebo group 24 (85.7%). Thus, the difference was not statistically significant (P value>0.05). On Day 16 and Day 30, all patients with mild and moderate category recovered in both groups.

Time to Improvement of Clinical Symptoms

Overall, patients receiving Cordyceps had mean improvement of clinical symptoms earlier than the patients receiving Placebo (6.6 ± 2.8 days *Vs* 7.0 ± 3.3 days), though the difference was not statistically significant (P value>0.05). In mild category, patients receiving cordyceps had symptoms improved a day earlier as compared to patients receiving placebo (6.6 ± 2.9 days Vs 7.3 ± 3.8 days), though the difference was not statistically significant (P value>0.05). In moderate category, the mean time to improvement of symptoms was 6.0 ± 2.6 days in Cordyceps group and 6.0 ± 2.1 days in Placebo group. The difference was not statistically significant (P value>0.05).

Time to Recovery of Clinical Symptoms

The mean time to recovery of symptoms were comparable between Cordyceps and Placebo groups. Similarly, mean time to recovery of symptoms were comparable between Cordyceps and Placebo groups for mild and moderate category patients.

Status of RT-PCR at Day 10

Proportionately higher number of patients showed RT-PCR negative result in Cordyceps group 17 (56.7%) as compared to Placebo group 13 (46.4%) on Day 10, though the difference was not statistically significant (P value>0.05).

Length of Hospital stay:

The mean length of hospital stay in moderate category patient was 14 ± 3.7 days in Cordyceps group and 11 ± 3.2 days in Placebo group. Difference in the hospital stay in two groups was statistically not significant.

Serum biomarkers

There was no significant change in the mean values of IL-6, ferritin, LDH, CRP and D-dimer levels at Day 5 and Day 10 as compared to Day 1 values in patients receiving Cordyceps capsules. Also, the comparison in between the patients receiving Cordyceps capsules and Placebo capsule was not significant statistically. There was significant change in the mean values of MCPIP, CXCL10 and IL-1β levels at Day 5 and Day 10 as compared to Day 1 values in patients receiving Cordyceps capsules. However, this change was seen in Placebo group also. The comparison in between the patients receiving Cordyceps capsules and Placebo capsule was not significant statistically except for CXCL10 where the comparison was significant at Day 10. Significant changes were seen in biomarkers CRP and CxCL10 in moderate category patients at Day 5 and Day 10 respectively. The mean IgG levels at Day 16 was 26.86 ± 27.45 in Cordyceps group and 16.52 ± 22.86 in Placebo group. There was no statistical significance between the values of two group.

TEAEs

Overall, 10 (17.2%) patients developed 16 treatment emergent adverse events (TEAEs). Out of these, 12 TEAEs were reported in 6 (20.0%) patients receiving Cordyceps capsules and 4 TEAEs were reported in 4 (14.3%) patients receiving placebo capsules. Nine TEAEs were treatment related. Of these, 5 TEAEs were related to Cordyceps capsules and 4 TEAEs were related to Placebo capsules. 3 (10.0 %) patients in Cordyceps group and 4 (14.3%) patients in Placebo group reported the related TEAEs. None of the patients in the study developed severe and serious TEAEs. Two patients had drug interruption due to progression of disease to moderate category. Overall, incidence of TEAEs were minimal. The reported TEAEs belongs to the gastrointestinal system followed by General disorders and administration site conditions and Nervous system disorders. Gastrointestinal TEAEs were common in patients who received Cordyceps capsule as well as the Placebo capsules.

Laboratory assessment:

The haematology parameters in Cordyceps group did not change significantly at Day 5 and Day 16 from the Day 1 visit except WBC count, platelet count and eosinophil count. These counts changes significantly at Day 16 as compared to Day 1 values but does not have any clinical significance. The biochemistry parameters in Cordyceps group did not change

significantly at Day 5 and Day 16 from the Day 1 visit except ALP, ALB and BUN. Though these values changed significantly at Day 5 and Day 16 as compared to Day 1 values, but it does not have any clinical significance.

Summary

On the basis of the *in-silico* study of the nucleosides present in C. *militaris,* it can be concluded that they may be effective in the treatment of SARS-CoV2 by following mechanism similar to that of the tested drug remdesivir i.e. RdRp inhibition. Overall theoretical and literature analysis of the key phytoconstituents of this marketed fungal formulation suggest that it may be imperative to explore it for the extended therapy in COVID-19. Since this product is already in market with no significant toxicity (as reported in the literature) , investigator suggests Ambrosia food farm.co for its preclinical and subsequent clinical evaluation as an 'add on therapy' in COVID patients owing to immense urgency to manage this global pandemic situation. Cordyceps capsules given at a dose of 500 mg three times a day along with supportive treatment showed effectiveness in patients with mild to moderate Covid-19 infection as evident by proportionately higher number of recoveries at Day 5, relatively shorter time for improvement of clinical symptoms, proportionately higher number of patients showing negative RT-PCR test on Day 10 and significant change in biomarkers such CRP, CxCL10 and IL-1β on day 5 and 10 as compared to baseline. Cordyceps capsules given at a dose of 500 mg three times a day along with supportive treatment showed effectiveness in patients with mild to moderate Covid-19 infection as evident by proportionately higher number of recoveries at Day 5, relatively shorter time for improvement of clinical symptoms, proportionately higher number of patients showing negative RT-PCR test on Day 10. Significant changes were seen in biomarkers MCPIP, CxCL10 and IL-1β for overall (both mild and moderate patients) on Days 5 and 10 as compared to baseline; and in biomarkers CRP and CxCL10 in moderate category patients at Day 5 and Day 10 respectively. Recovery of symptoms was mainly seen in mild patients, wherein patients receiving cordyceps had symptoms improved a day earlier as compared to patients receiving placebo (6.6 days Vs 7.3 days). The statistical significances could not be reached between group comparisons with Placebo for various parameters, due to limited sample size in this signal seeking study. No significant worsening of the disease related markers such as CRP, IL-6, ferritin, and D-dimers signifying that the disease was not worsened in patients who received Cordyceps, further confirming that the disease severity remained stable and did not worsened over a period. Thus, this proof of concept, signal seeking study showed the role of Cordyceps as an add on therapy in the treatment of patients with mild to moderate Covid-19 infection. Cordyceps at a dose of 500 mg three times a day for 15 days were safe and well tolerated in patients with mild to moderate Covid-19 infection. The reported TEAEs were mild to moderate in severity and were managed with/without medications. No meaningful drug related changes were observed in vitals, haematology, biochemistry, urinalysis, and ECGs in patients receiving Cordyceps capsules and changes were similar to Placebo. None of the patients in the study had severe or serious TEAEs. There was no drug interruption and dose reduction due to adverse events any of the patient. This is to be expected, based on the known safety profile of the active ingredients and their long history of use of cordyceps in humans.

Conclusion

Cordyceps at a dose of 500 mg three times a day for 15 days were safe and well tolerated in patients with mild to moderate Covid-19 infection. The reported TEAEs were mild to moderate in severity and were managed with/without medications. No meaningful drug related changes were observed in vitals, haematology, biochemistry, urinalysis, and ECGs in patients receiving Cordyceps capsules. None of the patients in the study had severe or serious TEAEs. There was no drug interruption and dose reduction due to adverse events in any of the patient. Overall, the results from present study are encouraging and offers Cordyceps as a safer and effective

add on therapy to standard of care treatment in patients with mild to moderate Covid-19 infection. However, the results require cautious interpretation. In the absence of the statistical power required to draw a strong conclusion, the findings of this early signal-seeking study need to be verified in a larger population study.

Bibliography

1. WHO, Coronavirus disease 2019 (COVID-19) situation report—163. July 2020. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200701-covid-19-sitrep-163.pdf?sfvrsn=c.
2. Lin BQ. and Li SP. Cordyceps as an herbal drug (Vol. 5), chapter. 2011.
3. Kim HG, Shrestha B, Lim SY, et al. Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF-κB through Akt and p38 inhibition in RAW 264.7 macrophage cells. European journal of pharmacology. 2006;545(2-3):192-199.
4. Shin S, Moon S, Park Y, et al. Role of cordycepin and adenosine on the phenotypic switch of macrophages via induced anti-inflammatory cytokines. Immune Network. 2009;9(6):255-264.
5. Panicali DL. and Nair CN. Effect of cordycepin triphosphate on in vitro RNA synthesis by picornavirus polymerase complexes. J Virol. 1978;25(1):124-128.
6. Sung SC. Inhibitors of RNA and DNA biosynthesis. Metabolic Inhibitors V3: A Comprehensive Treatise. 2012;175.
7. Chen M. Cheung FW. Chan MH. et al. Protective roles of Cordyceps on lung fibrosis in cellular and rat models. J. Ethnopharmacol. 2012;143(2):448-454.
8. Hui PK. and Chan MH. Cordyceps sinensis improved post-SARS pulmonary fibrosis. Hua Xia Medical Journal (Hong Kong). 2006;3:172–176.
9. Parvez MSA, Azim KF, Imran AS, et al. Virtual Screening of Plant Metabolites against Main protease, RNA-dependent RNA polymerase and Spike protein of SARS-CoV-2: Therapeutics option of COVID-19. arXiv preprint arXiv:2005.11254. 2020.
10. Lee HH, Park H, Sung GH, et al. Anti-influenza effect of Cordyceps militaris through immunomodulation in a DBA/2 mouse model. J. Microbiol. 2014;52(8):696-701.
11. Ohta Y, Lee JB, Hayashi K, et al. In vivo anti-influenza virus activity of an immunomodulatory acidic polysaccharide isolated from Cordyceps militaris grown on germinated soybeans. J.Agr.Food.Chem. 2007;55(25):10194-10199.
12. Wang N, Li J, Huang X, Chen W. and Chen Y. Herbal medicine Cordyceps sinensis improves health-related quality of life in moderate-to-severe asthma. Evid-Based Compl Alt. 2016.
13. Wang NQ, Jiang LD, Zhang XM. and Li ZX. Effect of dongchong xiacao capsule on airway inflammation of asthmatic patients. Zhongguo. Zhong. Yao. Za. J. Chinese Materia Medica. 2007;32(15):1566-1568.
14. Shen ZK. Therapeutic effect observation of Bailing capsule and medical gymnastics to COPD in paracmasis. Zhongguo. Zhong. Yao. Za. J. Chinese Materia Medica. 2008;33(8):942-944.
15. Huang DH, Wu L, He DP. and Lin L. 2005. Clinical observation of tranquilization period of chronic obstructive pulmonary disease treated by integration of traditional Chinese medicine and Western medicine. J Fourth Mil Med Univ. 2005;26(17):1611-3.
16. YU HB, LIU YF. and WU LX. Acupuncture combined with music therapy for treatment of 30 cases of cerebral palsy. J.Tradit. Chin. Med. 2009;29(4):243-248.
17. Yang L, Jiao X, Wu J, et al. Cordyceps sinensis inhibits airway remodeling in rats with chronic obstructive pulmonary disease. Exp. Ther. Med. 2018;15(3):2731-2738.

18. Sun X, Feng X, Zheng D, et al. Ergosterol attenuates cigarette smoke extract-induced COPD by modulating inflammation, oxidative stress and apoptosis in vitro and in vivo. Clin. Sci. 2019;133(13):1523-1536.
19. Yan W, Li T. and Zhong Z. Anti-inflammatory effect of a novel food Cordyceps guangdongensis on experimental rats with chronic bronchitis induced by tobacco smoking. Food & Function. 2014;5(10):2552-2557.