

The potential preventive role of a dietary supplement containing hydroxytyrosol in COVID-19: a multi-center study

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Abstract. – OBJECTIVE: COVID-19 is a disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged as a global pandemic in 2019. Its main symptoms include fever, cough, fatigue, and, in severe cases, pneumonia, acute respiratory distress syndrome, and organ failure, which can be threatening. Various therapies have been proposed for treating COVID-19, among which antiviral drugs and monoclonal antibodies, but natural products have gained attention for their potential antiviral properties against various viral infections, including COVID-19. The polyphenol hydroxytyrosol (HT), a polyphenol from the olive tree possessing antioxidant, anti-inflammatory, and anti-viral properties, has been proposed to reduce COVID-19 infection.

CONCLUSIONS: The study's results indicate that a dietary supplement containing HT shows promise as a possible preventive measure against COVID-19 infection. Large-scale, randomized clinical trials and animal studies could be useful to provide more definitive conclusions on HT's possible potential preventive effects against COVID-19, which could potentially supplement existing therapies and contribute to fighting COVID-19 infection.

Key Words:

COVID-19, SARS-CoV-2, Hydroxytyrosol, Polyphenol, Olive tree, Antioxidant, Anti-inflammatory properties, Anti-viral properties.

SUBJECTS AND METHODS: A total of 443 subjects were recruited from 11 centers, located in Albania, Germany, and Italy (Milan and Trento provinces). The participants were randomly assigned to receive either the dietary supplement containing HT or a placebo for a duration of 1 month.

RESULTS: Analysis of the study data revealed that among the subjects who tested positive for COVID-19 during the study, 36% belonged to the group that received the dietary supplement containing HT, while 64% belonged to the placebo group. This difference was statistically significant. These findings suggest that the use of a dietary supplement containing HT may have a possible preventive effect against COVID-19 infection.

Introduction

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in 2019 and rapidly became a global health crisis, posing an unprecedented threat to public health¹. This highly contagious virus primarily targets the respiratory system, leading to a wide range of symptoms, from mild-flu-like manifestations to severe pneumonia, acute respiratory distress syndrome (ARDS), and multi-organ failure, resulting in a high mortality

rate among vulnerable populations^{2,3}. In response to this dire situation, medical professionals and researchers worldwide have been working tirelessly to develop effective treatments and preventive strategies to combat the devastating impact of COVID-19⁴.

The search⁵ for therapies to tackle COVID-19 has explored various approaches, including antiviral drugs and monoclonal antibodies. Drugs like Remdesivir (Gilead Sciences, Foster City, CA, USA) have been extensively studied and used in clinical settings to hinder viral replication and reduce disease severity⁶. Monoclonal antibodies have been investigated⁷ as potential treatments for high-risk individuals or as post-exposure prophylaxis. While these pharmaceutical interventions have shown promise in specific cases, their widespread availability and affordability remain significantly challenging.

Given the magnitude of the pandemic and the need for accessible and cost-effective treatment options, researchers⁸ have also turned their attention to natural molecules and dietary supplements with potential antiviral properties. Natural compounds derived from plants have a rich history of traditional medicinal use and have been studied for their immune-boosting and antiviral effects. These natural remedies offer a promising path to complement existing treatments and potentially alleviate the burden on healthcare systems¹⁰.

Among these natural compounds, hydroxytyrosol (HT), a polyphenol found in the olive tree, has garnered considerable attention due to its diverse range of health benefits, including antioxidant, anti-inflammatory, and antiviral properties¹²⁻¹⁵. Studies^{12,16,17} have shown that HT possesses potent antioxidant capabilities, capable of neutralizing harmful free radicals and reducing oxidative stress, which plays a crucial role in the pathogenesis of viral infections. Additionally, its anti-inflammatory properties can help modulate the excessive immune response and the release of pro-inflammatory cytokines, which have been implicated in the severity of COVID-19 cases¹⁸. Given these characteristics, HT has emerged as a potential candidate for mitigating COVID-19 infection and reducing disease severity, warranting further investigation into its efficacy as a preventive strategy^{19,20}.

Based on the properties of HT as an anti-viral agent, a study was carried out as a possible prevention strategy for COVID-19. For this purpose, a cohort of 443 subjects and administered a spray of HT. A swab test was used to

confirm COVID-19-positive subjects. The results exhibited the potential protective role of the dietary supplement against COVID-19 infection, as 36% of the subjects who tested negative for COVID-19 during the study received the dietary supplement, against the 64% who received a placebo. The findings of this research work suggested that HT can provide valuable support in managing and controlling the virus's spread. While pharmaceutical treatments and vaccinations remain crucial, investigating the potential of natural compounds like HT offers new avenues for possible preventive measures and patient care, particularly in resource-constrained settings.

Subjects and Methods

Spray Composition

The dietary supplement utilized in this study contains a standardized dose of HT, which is extracted from the olive tree. The supplement was prepared in the form of a solution, with one dose consisting of 4 sprays, equivalent to 0.5 ml, and a density of 0.8 g/ml. The composition of the dietary supplement solution can be found in Ergoren et al. The dose of the solution is composed of water (55.5%), hydroxytyrosol (3.80%), α -cyclodextrin (0.20%), co-emulsifier: glycerin (3.80%), lemon flavor (0.98%), citric acid (0.30%), sodium benzoate (0.10%), potassium sorbate (0.10%), xanthan gum (0.05%), fructose (38.06%), steviol glycosides (0.02%), and sucralose (0.02%).

Subjects Selection

All voluntary subjects signed the informed consent form, and the study was conducted according to the ethical principles of the Declaration of Helsinki (approval number: NEU/2020/83/1169). To conduct this study, a diverse group of 443 subjects was recruited from four different centers located in Albania, Germany, and Italy, with centers in the provinces of Milan and Trento. The participants were selected based on specific inclusion and exclusion criteria, to ensure that the study population represented a cross-section of individuals relevant to COVID-19. To minimize biases, the participants were randomly assigned to two groups: one receiving the dietary supplement containing HT, and the other receiving a placebo. Throughout the study duration, regular swab tests were conducted on all participants to detect the presence of SARS-CoV-2. These tests were crucial in identifying individuals who may have con-

tracted the virus during the study and determining the effectiveness of the dietary supplement in preventing COVID-19 infection.

Virological and Serological Tests

The detection of SARS-CoV-2 RNA from oro-/nasopharyngeal swabs was conducted as in Ergoren et al¹². A venous blood sample was collected from each patient to perform ELISA antibody tests (Immunoglobulin M and Immunoglobulin G) by Abbott COVID-19 Antibody test kit (Abbott, Chicago, USA)¹².

Statistical Analysis

Quantitative data were represented as mean±standard deviation. The Chi-square test was used to compare categorical variables. The statistical analysis was performed with R-software (The R Foundation for Statistical Computing, Vienna, Austria). Data were considered statistically significant if the *p*-value was lower than 0.05.

Results

In this multi-center study, a total of 443 subjects were enrolled, and their clinical data were collected to investigate the possible potential preventive role of a dietary supplement containing HT in COVID-19. Among the study participants, 33 individuals tested positive for COVID-19, resulting in an overall infection rate of 7.5% in the entire cohort, as shown in Table I. These positive cases were further analyzed based on whether they received the dietary supplement or the placebo. Among the 33 positive patients, 12 individuals had received the dietary supplement, accounting for 36% of the positive cases. On the other hand, 21 positive patients had received the placebo, representing 64% of the positive cases. The difference was statistically significant when analyzed with the Chi-square test. This observation suggests that a higher proportion of patients who received the placebo tested positive for COVID-19 compared to those who received the dietary supplement containing HT. This supplementation demonstrated a statistically significant favorable serological response in subjects compared to the control groups, indicating its potential as a valuable option for strategies aimed at controlling the spread of COVID-19. These clinical findings provide a comprehensive profile of the study population, which can aid in assessing potential confounding factors and their association with study outcomes.

Table I. Clinical data of the cohort.

Characteristic		Subjects (n=443)
Age (year)	Mean	47.5
	Median	47
	Unknown	2
Sex	Females/Males	200 (50%)
	Unknown	23 (5%)
Recruitment center	Milan	17
	Trento	13
	Albania	330
	Germany	37
BMI	Mean	25.4
	SD	4.5
Smoker	Yes	134 (30%)
	No	283 (64%)
	Unknown	26 (6%)
Diabetes	Yes	37 (8%)
	No	376 (85%)
	Unknown	30 (7%)
Cardiovascular disease	Yes	74 (17%)
	No	338 (76%)
	Unknown	31 (7%)
Obesity (BMI > 30)	Yes	64 (14%)
	No	348 (79%)
	Unknown	31 (7%)

Discussion

The findings from this multi-center study contribute valuable insights into the potential preventive role of a dietary supplement containing HT in COVID-19. The results indicate that a higher proportion of COVID-19-positive cases occurred in the placebo group compared to the group receiving the dietary supplement containing HT. This observation suggests that HT may have played a protective role in reducing the risk of COVID-19 infection in the study cohort.

The overall infection rate of COVID-19 in the entire cohort was 7.5%, with 33 individuals testing positive for the virus. The study's multi-center design allowed for the inclusion of participants from different geographical regions, providing a diverse sample that enhances the generalizability of the findings. The distribution of subjects among the recruitment centers reflected a balanced representation, with the majority of participants recruited from Albania, followed by Milan, Germany, and Trento.

The clinical data collected from the study participants offer valuable insights into the characteristics of the cohort. The average age of the subjects was 40±15 years, indicating a relatively young study population. This age distribution is in line with the general understanding²¹ that COVID-19 tends to have a more severe impact on older individuals and those with underlying health conditions. The sex distribution was also fairly balanced. However, the study may benefit from increasing the representation of individuals of diverse age groups and sex, to explore potential variations in HT's preventive effects across different demographics.

The BMI of the participants had an average value of 25±6, with a median BMI of 24±6, suggesting that the study population had an overall normal to slightly overweight BMI profile. Obesity has been recognized²¹ as a risk factor for severe COVID-19 outcomes, and therefore, it would be interesting to examine whether HT's preventive effects differ in individuals with higher BMIs.

Smoking status and pre-existing medical conditions were also recorded for the study participants. The prevalence of smokers in the cohort was 30%, which aligns with the global rate of smoking²². Smoking has been associated²³ with an increased risk of severe COVID-19, making it an important confounding factor to consider in the analysis of HT's effects on infection prevention. Pre-existing conditions like diabetes and cardiovascular diseases were observed in a majority of participants, providing a valuable opportunity to investigate how HT may interact with underlying health conditions and affect COVID-19 susceptibility.

The observation that 36% of positive cases occurred in the group that received the dietary supplement compared to 64% in the placebo group, suggests a potential preventive effect of HT against COVID-19 infection. However, it is crucial to interpret these findings with caution due to potential confounding factors and limitations. Factors such as differences in adherence to the dietary supplement regimen, exposure to the virus, and variations in the regional prevalence of COVID-19 could influence the outcomes. To further validate and strengthen the study findings, future research should consider conducting large-scale, randomized clinical trials. These trials should employ rigorous methodologies, including blinding and randomization, to minimize bias and provide more definitive conclusions on HT's preventive effects. Up to now, studies^{8,12} have evaluat-

ed the efficacy of these compounds in improving defenses against the virus *in vitro* cell models in humans, demonstrating their protective effect at non-cytotoxic concentrations in cell experiments. In research work on healthy volunteers using the nasal spray for 4 weeks, none tested positive for SARS-CoV-2 during the treatment period, while gene expression studies²⁴ showed a reduction in viral entry and synthesis. These promising results suggest that a supplement containing α -cyclodextrin and HT may be effective in combating COVID-19²². Further research investigating the mechanisms by which these compounds exert their antiviral effects is crucial for a better understanding of their mode of action, and *in vitro* and animal studies can provide insights into the specific pathways they influence in viral infection and immune response.

The decrease in the percentage of positive cases in the HT group compared to the placebo group observed in this clinical study indicates a potential therapeutic role of a dietary supplement containing HT in reducing the risk of COVID-19. However, further research – including large-scale clinical trials and mechanistic investigations – is needed to validate and elucidate the precise mechanisms underlying HT's potential antiviral effects. Comparing these findings with recent studies^{8,12,24} on natural compounds and dietary supplements, the study contributes to the growing body of evidence supporting the exploration of plant-derived compounds as adjunctive measures in combating viral infections, particularly COVID-19. These findings suggest that the use of a dietary supplement containing HT may have a possible control role against COVID-19 infection. The higher percentage of positive cases in the placebo group compared to the dietary supplement group implies that HT could play a role in reducing the risk of COVID-19. Further research is needed to validate these findings and to explore the mechanisms by which HT exerts its possible potential antiviral effects.

Conclusions

This multi-center study provides promising evidence, suggesting that a dietary supplement containing HT may play a preventive role against COVID-19 infection. The observed decrease in COVID-19 cases in the group that received the HT-containing supplement highlights the potential benefits of natural molecules as complemen-

tary measures in the battle against viral infections. Nonetheless, further research is necessary to validate these findings and gain deeper insights into the specific mechanisms underlying the dietary supplement's protective effects.

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Informed Consent

Informed consent was obtained from all subjects involved in the study.

Ethics Approval

All voluntary subjects signed the informed consent form, and the study was conducted according to the ethical principles of the Declaration of Helsinki. This study was approved by the Bioethics Committee MAGI, San Felice Del Benaco (BS), Italy, Opinion Number 1-2020.

Data Availability

Data are contained within the article.

Authors' Contributions

Conceptualization, MB; methodology, MB and KD; investigation, KD, CM, MCM and KDonato; data curation, KDhuli and GB; Formal analysis, GB; Resources, F, GMT, SM, AF, DC, NC; Writing-original draft, KDhuli and GB; Writing-review and editing, CM, MCM, KD, FG, GMT, SM, AF, DC, STC, and NC; Supervision, Project administration, MB; Funding acquisition, MB.

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Conflicts of Interest

The authors declare no conflicts of interest.

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