

Individual Participant Data (IPD) Sharing Statement

Will individual participant Data be available (including data Dictionary)	Yes
What data in particular will be shared?	All the individual participant data that underlie the results reported in this article, after deidentification
What other documents Will be available?	Study protocol, statistical analysis plan, clinical study report
When will data be Available (start and end dates)	Immediately following publication, No end date
With whom?	Researchers who provide a methodologically sound proposal
For what types of Analyses?	To achieve aims in the approved proposal
By what mechanism Will data be made available?	Data are available indefinitely in the journal after publication

Quercetin as an antiviral agent against Coronavirus infection: first Double-blind clinical trial in Tunisia

Marwa Gargouri^{a,b}, H  la Gargouri^c, JihenJedidi^d, NaouesIssaoui^e, Imen Ketata^f, ,Houda Ghorbel^a, Leila Zrelli^a,
Hajer Azouzi^a, Mohamed Ali Boukhchina^g

- a. Department of Infectious Diseases, Mohamed Sassi University Hospital, Gabes, Tunisia
- b. Laboratory of Resarch, toxicology, environmental microbiology and Health (LR17ES06), Faculty of Sciences of Sfax, University of Sfax, Sfax, Tunisia
- c. Center of Biotechnology of Sfax, Laboratory of Molecular and Cellular Screening Processes, Sfax, Tunisia
- d. Epidemiology Department, Hedi Chaker Hospital, Sfax, Tunisia,
- e. Department of Otolaryngology, Mohamed Sassi University Hospital, Gabes, Tunisia
- f. Department of Neurology, Mohamed Sassi University Hospital, Gabes, Tunisia
- g. Health and Drug corporation HDC Laboratory, Ben Arous, Tunisia

Corresponding author: H  la Gargouri. Center of Biotechnology of Sfax, Laboratory of Molecular and Cellular Screening Processes, University of Sfax, 3018 Sfax, Tunisia E-mail: hela.gargouri@usf.tn Tel: +216 22 36 41 41 orcid: **0000-0002-3275-1816**

Materials and methods:

We conducted a prospective experimental study of the clinical trial type involving two groups randomly divided: one receiving the quercetin treatment (800mg/day) for 10 days and then 400 mg daily for a month (Group Q) and the other receiving a placebo (Group P). The study plan was explained, and informed consents were obtained from the patients who agreed to participate in our study. Ethics Committee of Mohamed Sassi University Hospital approved the study.

- **Identification of patients with COVID-19, inclusion and exclusion criteria:**

In our study, samples were obtained from patients admitted to Mohamed Ben Sassi Hospital, Gabes, a governorate of southern Tunisia, during the period ranging from May 2021 to April 2022.

The patients with epidemiological history, fever and/or respiratory symptoms, presence of imaging findings of novel coronavirus pneumonia, and real-time reverse transcriptase-polymerase chain reaction (RT-PCR) fluorescence for SARS-CoV-2 nucleic acid which yielded positive results were included. Furthermore, patients who did not agree to participate in our study, patients with negative PCR report despite respiratory involvement were excluded.

The epidemiological data, the laboratory test result data, and radiological data were acquired from medical files. Exposure history, disease onset, and clinical features and prognostic factors of patients with COVID-19. The usage of antiviral drugs, corticotherapy, respiratory supplementary treatments, the length of intensive care unit (ICU) stay, complications, and comorbidities, were also recorded.

Inclusion criteria: Male or female, 20–88 years of age and diseased and non-diseased hospitalized subjects.

Exclusion criteria: pregnant women or lactating and non-hospitalized subjects.

Intervention Description:

Description of drug and placebo:

- Drug Group (Group Q): (800 mg quercetin/day (400 mg twice a day) for 10 days and then 400 mg daily for a month
- Placebo Group (Group P): The patients allocated to the placebo group received placebo daily for a month. Placebo supplements were prepared exactly the same way minus the quercetin.

The allocation of the patients in the two groups was made using the table of random numbers and resumed in a table containing the allocation of each patient.

Sealed envelopes containing one of the compared treatments were prepared in advance by the investigator for each patient. Thus, the patient and the attending physician were unaware of the treatment administered. Only the investigator was aware of the nature of the treatment administered.

Judgment criteria:

The primary judgment criterion was the reduction in complications and mortality related to the disease after one month post-intervention.

The secondary judgment criterion was the reduction in the duration of hospitalization.

- **Data collection and laboratory tests.**

Demographic data including age, sex, smoking habits, body temperature, and comorbidity data including hypertension, diabetes, and coronary heart disease were collected.

The hospitals utilized next-generation sequencing and real-time RT-PCR assay for the detection of COVID-19. Briefly, throat-swab specimens were obtained from patients with fever, cough, or dyspnea and subjected to PCR examination.

- **Statistical analyses:**

The calculation of the number of subjects necessary was based on a reduction in the rate of hospital mortality of at least 15%. This rate was estimated at 40% based on a review of rate mortality in our service on this moment. The minimum number of subjects required was 120 subjects in each group to have a power of 80%.

Data entry and analysis were done using SPSS software. The description of the qualitative variables was made by calculating the numbers and the percentages. The comparison of the frequencies was made by the Pearson chi 2 test when the theoretical numbers were ≥ 5 and by the Fischer test otherwise. For quantitative variables, description was made by calculating the mean and the standard deviation, the comparison of two means was made by the Student test after having checked the conditions of application. When these conditions were not verified, the non-parametric Mann Whitney test was used. The representation of the subgroups analysis was made by the Excel software.

The significance threshold used was 5%.

Results:

A total of 244 COVID-19 patients were included and randomized to one of two groups: Q-800 (800 mg quercetin / day) (group "Q") or placebo (group "P"): 122 in each group.

1. Participant characteristics:

The average age of quercetin group was 65.34(± 15.13)years and 67.41 (± 15.48) years in the placebo group. In both groups, the predominant gender was male. In group "Q", 59.8% were male versus 63.1% in group "P". The smoking rate was 38% in group "Q": vs. 33.6% group "P"; $p=0.48$.

No significant difference could be discerned between the two groups in baseline characteristics of age, gender, diabetes, arterial hypertension, cardiopathy disease ($p > 0.05$) (Table 1).

2. Characteristics of SARS-CoV-2 infection:

Characteristics of SARS-CoV-2 infection were described in table 2. No statistical significant difference between the 2 groups was found in the chest CT damage (0.37), white blood cells ($p=0.71$), C-reactive protein (CRP) ($p=0.61$) and D-dimer ($p=0.46$) (Table 2).

Concerning complications, Respiratory aggravation was higher in placebo group (21.3%) versus (9.0%) in Quercetin group, RR=0.42; CI95%: [0.22 – 0.81]; $p=0.007$. However, no significant difference was noted with other complications (cardiac complication, mortality rate and duration of hospitalization ($p > 0.05$)) (Table 3).

For complications and death, a subgroup analysis showed that the effect of quercetin was beneficial in subjects having less than 40% CT involvement (Figure 2).

In fact, among the 80 subjects with CT involvement $< 40\%$, 24 (30%) developed complications. Of these, 7 were in the treatment group (i.e. 18.4% of the Q group) and 17 were in the placebo group (i.e. 40.5% of this group), $p=0.03$; RR= 0.45; CI 95% [0.21 - 0.97] (figure 2).

Regarding cardiac complications, the subgroup analysis showed that the treatment was effective for subjects with a medical history, especially diabetes and hypertension (Figure 3).

In fact, in patients with a medical history ($N=166$), 4.4% of Quercetin-treated subjects had cardiac complications versus 14.0% of untreated subjects, RR=0.30; CI95%: [0.10 - 0.92]; $p=0.02$ (Table 4).

Author contribution

Marwa Gargouri (MG): Conceptualization, Resources, Writing - Review & Editing, Validation.

Hela Gargouri (HG): Conceptualization, Resources, Writing - Review & Editing.

Jihenjdidi (JJ): Participation in the drawing of tables and figures; Data analysis

Nawresissaoui (NI): Conceptualization, Resources,

Imen Ketata (IK): Writing, Review and editing

Houda Ghorbel (HG): Review and editing

Leila Zrelli (LZ): Review and editing

Hajer Azouzi (HA): Review and editing

Mohamed Ali Boukhchina (MB): Providing the Quercetin samples

All authors (MG, HG, JJ, NI, IK, HG, LZ, HA, MB) contributed to the research and approved the final manuscript.

Availability of data and materials

Data are available upon reasonable request. Dr Marwa Gargouri will be contacted by patients if needed.

Declarations

Ethical approval

The local research ethics committee of the University Hospital Mohamed Sassi of Gabes (Tunisia) approved this study.

Consent to participate

The study plan was explained, and informed consents were obtained from the patients who agreed to participate in our study. Ethics Committee of Mohamed Sassi University Hospital approved the study.

Consent for publication

All authors consented to the publication of this manuscript.

Competing interests

The authors declare no competing interests

Table 1: Participant characteristics

Participants' characteristics	Groups		p
	Quercetin group (N=122)	Placebo group (N=122)	
Age (years): mean (SD)	65.34 ($\pm$ 15.13)	67.41 ($\pm$ 15.48)	0.29
Gender(%)	Male : 73 (59.8) Female : 49 (40.2)	Male : 77 (63.1) Female : 45 (36.9)	0.59
Smoking(%)	46 (38.0)	39 (33.6)	0.48
Comorbidity : (%)	90 (73.8)	76 (62.3)	0.055
Arterial hypertension(%)	43 (35.2)	47 (38.5)	0.59

Diabetes(%)	42 (34.4)	37 (30.3)	0.49
Cardiopathy diseases(%)	24 (19.7)	20 (16.4)	0.5
Renal failure(%)	13 (10.7)	14 (11.5)	0.83
Hypothyroidia(%)	7 (5.7)	6 (4.9)	0.77
Dyslipidemia(%)	6 (4.9)	6 (4.9)	1
Respiratory diseases(%)	10 (8.2)	6 (4.9)	0.3
Neoplasia(%)	2 (1.6)	2 (1.6)	1

Table 2: Characteristics of SARS-CoV-2 infection

Participants' characteristics		Groups		p
		Quercetin group (N=122)	Placebo group (N=122)	
Oxygen saturation in ambient air: mean, (SD)		79.13 ($\pm$ 10.26)	77.85 ($\pm$ 12.00)	0.37
Chest CT	<40%(%)	38 (38.4)	42 (44.7)	0.37
	$\geq$ 40%(%)	61 (61.6)	52 (55.3)	
White blood cell (/L): (mean, SD)		8955.5 ($\pm$ 4531.2)	9656.6 ($\pm$ 6656.9)	0.33
C-reactive protein (mg/L): (mean, SD)		124.23 ($\pm$ 83.9)	124.26 ($\pm$ 84.4)	0.99
D-dimer (ng/ml): median, (SIQR)		969.6 (896.0)	1100 (1349)	0.49*
Non-invasive ventilation(%)		32 (26.2)	36 (29.5)	0.56
Intubation(%)		2 (1.6)	2 (1.6)	1.00

*Mann Whitney test

Table 3: the main judgment criteria

	Groups		p
	Quercetin group(N=122) N (%)	Placebo group(N=122) N (%)	
Complications			
Cardiac complications	4 (3.3)	11 (9)	0.06
Neurological complications	1 (0.8)	2 (1.6)	1
Digestive complications	0	1	1
Aggravation renal function	0	1	1
Diabetes decompensating	1 (0.8)	4 (3.3)	0.37
Pulmonary embolism	14 (11.5)	15 (12.3)	0.84
Thromboembolic complications	15 (12.3)	16 (13.1)	0.84
Respiratory aggravation	11 (9.0)	26 (21.3)	0.007
Mortality	43 (35.2)	50 (41)	0.35
Duration of hospitalization (days): mean, (SD)	11.22 ($\pm$ 8.24)	10.42 ($\pm$ 5.76)	0.37

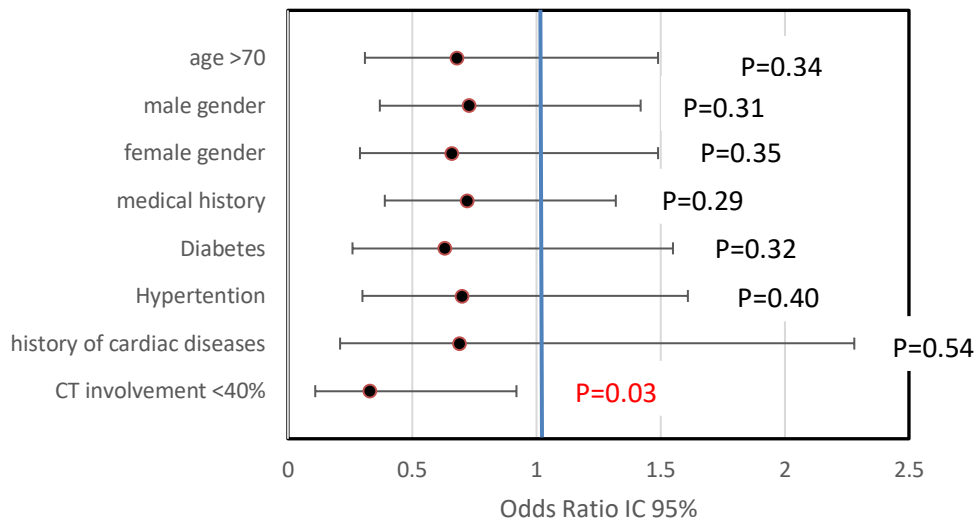


Figure 2: Subgroup study concerning complications and mortality

Table 4: Subgroup study of cardiac complications

	Total cardiac complications (%)	Quercetin group (%) [*]	Placebo group (%) [*]	p	RR	IC _{95%} RR
patients with a medical history (N=166)	15/166 (9.0)	4/90 [*] (4.4)	11/76 [*] (14.5)	0.02	0.30	[0.10 – 0.92]
Hypertension (N=90)	11 (12.2)	1/43 [*] (2.3)	10/47 [*] (21.3)	0.006	0.10	[0.01 – 0.81]
Diabetes (N=79)	7 (8.9)	1/42 [*] (2.4)	6/37 [*] (16.2)	0.04	0.14	[0.02 – 1.16]
Subjects with a history of cardiac	8 (18.2)	2/24 [*] (8.3)	6/20 [*] (30.0)	0.06	0.27	[0.06 – 1.22]

disease (N=44)						
----------------	--	--	--	--	--	--

*: complications in the group/ number of subjects included in the group

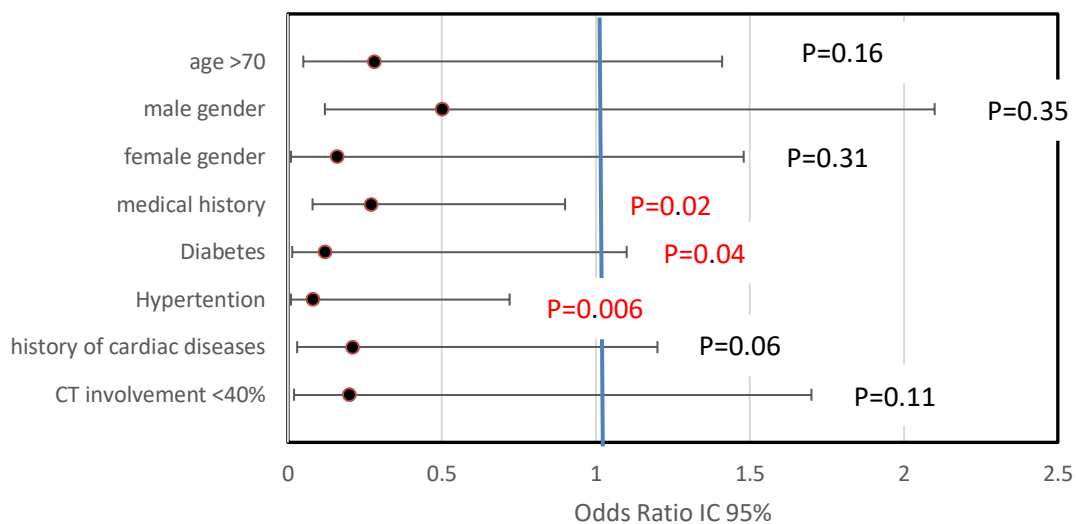


Figure 3: Subgroup study concerning Cardiac complications