Ministry of Education
Superior, Research
Scientific and Innovation

Phase II, open-label study to evaluate the safety, tolerance and efficacy of PAPIVIRINE in adults with COVID-19 in Burkina Faso

(API-COVID-19)

Study report 15 09 2021

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1. INTRODUCTION AND STATEMENT OF THE PROBLEM

In December 2019, a new coronavirus, now known as SARS-CoV-2, caused a series of acute atypical respiratory illnesses in Wuhan, Hubei province, China. The disease caused by this virus has been called COVID-19 (1).

This disease, transmitted from person to person, spreads mainly through the respiratory tract through salivary droplets, respiratory secretions and direct contact (2) for a low infectious dose (3).

First reported on December 31, 2019, as an epidemic (4), COVID-19 was declared as a pandemic on March 11, 2020 by the World Health Organization (WHO).

As of July 2, 2021, statistics showed a total of 183 million cases of COVID-19 worldwide, including more than 3 million deaths (5)

In this pandemic context, Burkina Faso notified its first case of COVID-19 on March 9, 2020 (6). As of July 2, 2021, a total of 13,479 cases have been identified, including 168 deaths (7).

The lack of specific therapy continues to be a problem (1), despite many avenues proposed by many teams. These tracks have indeed raised mixed hopes in the world (8-12).

Thus, the vaccine path seemed promising, pushing the international scientific community to “rush” in their uses, given the global health emergency caused by COVID-19 (3,7,11).

To date, their efficacy is constantly reassessed and their harmlessness is truly unknown at this stage, forcing users to a constant estimate (risk / benefit) or sometimes to more or less objective reluctance.

However, the World Health Organization (WHO) and the African Centers for Disease Control and Prevention (Africa CDC), aware of the enormous possibilities offered by traditional medicine, have recommended the search for a solution, in the face of this pandemic, also by exploiting this medicine (13).

Thus, like Madagascar, several states on the continent have pleaded for the integration of traditional medicine in the response against the pandemic, in particular the Congo, Cameroon, Gabon and Burkina Faso (12).

Since then, researchers, doctors, traditional healers and even African religious have been working with particular enthusiasm to find preventive and / or curative solutions in the face of COVID-19 (14).

It is in this context of health emergency that a clinical study was initiated in April 2020 aimed at evaluating the tolerance, safety and efficacy of the phyto-drug called APIVIRINE, developed in Benin from aqueous extracts of leaves of Dichrostachys glomerata.

Drug presentation

APIVIRINE is an antiretroviral phytomedicine proposed for the management of HIV / AIDS. It is made from the aqueous extracts of the leaves of Dichrostachys glomerata. It is presented in the form of 10 capsules of size n ° 2 of burgundy red color in blister packs (PVC, aluminum) in boxes of 40. APIVIRINE capsules are dosed at 350 mg of extracts rich in tannins, flavonoids, in saponosides, terpenes and steroids.

According to the WHO classification (2001; 2003) and the decree

N ° 2004-569 / PRES / PM / MS / MCPEA / MCEV / MESSRS authorizing the marketing of drugs from the traditional pharmacopoeia of Burkina Faso, APIVIRINE is classified in category III.

Figure 1: presentation of the APIVIRINE packaging

2. API-COVID-19 STUDY PROTOCOL

2.1. Goals

2.1.1. Primary objective

To assess the tolerance, safety and efficacy of the phytomedicine APIVIRINE in adult patients with COVID-19 without signs of severity

2.1.2. Secondary objectives

a. To assess the tolerance and safety of the phytomedicine APIVIRINE in adult patients with COVID-19 without signs of severity, in particular:

• Immediate IR reactions (reactions within 60 minutes of oral administration of the drug with emphasis on allergic reactions),

• During administration, any adverse effect noted or reported by the participant

• Any serious adverse reaction (SAE) that occurred between inclusion and leaving the study. The relationship between SAEs with the test drug should be established by the investigator, who will use the following definitions: unbound, possible binding, probable binding, established binding. Cardiac adverse events by performing an ECG in particular prolongation of the QT interval (> 500 ms).

• Biological tolerance within 1 day (between D1 to D21) following the first administration, compared with the basic data before the first dose by measuring the following biological parameters:

- the blood count: Number of red blood cells, hemoglobin level, hematocrit, VGM, TCMH, CCMH, number of platelets, number of white blood cells, Sedimentation rate.

- biochemistry: transaminases (ASAT, ALAT), creatinine, glycemia, C reactive protein (CRP).

b. To determine the effectiveness of the phytomedicine APIVIRINE on the viral load in adults with COVID -19 without signs of severity

• Proportion of patients with negative RT-PCR on D4, D7, D14, D21

• Viral load clearance time based on RT-PCR Cycle Threshold (CT);

• Time to clearance of fever and clinical signs present at patient admission.

c. To determine the acceptability of APIVIRINE in adult patients with COVID-19 without signs of severity:

• Acceptability of treatments in adult patients with COVID-19

• Proportion of adverse reactions / events of APIVIRINE

• Nature and severity of the side effects / adverse events of APIVIRINE

2.2. Hypothesis

Treatment with APIVIRINE is effective and tolerated in the management of patients with COVID-19 without signs of severity.

2.3. Study materials and methods

2.3.1. Type and duration of the study

This was an open-label phase II clinical study which evaluated the safety and efficacy of APIVIRINE in patients with COVID-19. Each patient was followed until recovery. The inclusion took place from October 30, 2020 to January 04, 2021. The total duration of the study was 10 weeks.

2.3.2. Study population

The study was carried out in patients without discrimination of sex whose age was between 20 and 65 years and positive for the coronavirus SARS-CoV-2 without signs of severity.

2.3.3. Inclusion criteria

The following patients were included in the study:

- Diagnosed positive at the screening sites by RT-PCR without signs of seriousness

- Aged at least 20 and at most 65

- Having signed the informed consent

- Being willing and able to comply with the study protocol for the duration of the study.

2.3.4. Non-inclusion criteria

Patients were not included in the study:

- With renal failure

- With heart failure

- Showing QTc interval > 500ms

- Diabetics with complications

- With immunodeficiency

- Having chronic liver disease with transaminases greater than 5 times normal

- Carriers of known chronic diseases (cancers, Tuberculosis, ...)

- Pregnant or breastfeeding females

- Participating in any other ongoing clinical study

- Adults unable to comply with the study protocol

- Presenting other conditions which, in the opinion of the investigator, would endanger the safety or rights of the study participant or render the subject unable to comply with the protocol

- Showing symptoms, physical signs of any disease that may interfere with the interpretation of the results of the study or compromise the health of the volunteers.

2.3.5. Study framework

- Patients were recruited from COVID-19 screening sites in Ouagadougou

- The patients were followed on an outpatient basis (home confinement), in accordance with the national guidelines for the management of simple cases of COVID-19 in force (national guidelines for the management of cases of coronavirus disease: COVID-19, February 2020).

- The national influenza reference laboratory (LNR-G) of the Institute for Research in Health Sciences (IRSS), carried out diagnostic tests for COVID-19 by RT-PCR in Ouagadougou.

2.3.6. Sample size

The open-label clinical trial considered a cohort of patients to whom APIVIRINE was administered with a repeated dose during the treatment period.

Under the assumption of an approximation between the binomial distribution and the reduced standard normal distribution and the assumption that treatment with APIVIRINE produces a cure rate greater than 80% after 14 days of treatment, the sample size minimum necessary to test this hypothesis was obtained by applying the following formula:

$$n = 2\left(\frac{Z_{1-\alpha} - Z_{1-\beta}}{\phi(\pi) - \phi(\pi_0)}\right)^2$$

in which :

- ✓ α = 5% the significance level,
- ✓ Z1 - α is the fractile of order 1- α
- ✓ 1- β is the power of detection of a difference between the healing proportions is set at 80%,
- ✓ Z1- β is the fractile of order 1- β of the reduced centered normal law,
- ✓ ϕ is the distribution function of a reduced centered normal distribution.
- ✓ ϕ (π) - ϕ (π o) is the effect size considered, i.e. the difference between the proportion n-ode cure of patients treated according to the national protocol and the rc proportion of cure of patients treated with APIVIRINE after one week of treatment.

Considering an objective of detecting an average effect according to Cohen's criterion, the minimum sample size determined using the values below was 41 patients. Considering a 10% non-response rate, a sample of 45 patients was included in the study.

2.3.7. Procedure for clinical and biological monitoring of patients

All 45 patients meeting the inclusion criteria underwent complete clinical and laboratory examinations at inclusion after giving their informed consent.

a. Activities at the first visit or inclusion visit (V1: D1)

• Informed consent

• Clinical parameters

Anthropometric parameters such as weight, height were measured.

Vital signs such as temperature, pulse, blood pressure, respiratory rate, pulsed oxygen saturation (SPO2), and heart rate were taken.

Medical and surgical history; respiratory functional signs such as the presence of a cough and dyspnea as well as other symptoms of COVID-19 were also looked for.

The complete physical examination of each patient was performed by a clinical physician

• **Paraclinical parameters**

Cardiac: An ECG was performed (with 12 leads) to look for a cardiac abnormality, in particular a prolongation of the QT interval (> 500 ms).

Pulmonary: computed tomography (CT) was performed

Biological: carried out from a blood sample taken on D1 before starting the treatment: blood count (CBC), VS, **Biochemical**: the determination of transaminases (ALT, AST), serum creatinine, blood glucose and the C- Reactive Protein (CRP) has been carried out

Blood culture was planned for relapsing fever after symptomatic treatment.

• **Co-morbidities:** all clinical or therapeutic conditions favoring the unfavorable evolution of the infection were sought.

b. Activities at the 2nd follow-up visit (V2: D4)

• Clinical parameters

Anthropometric parameters such as weight were measured.

Vital signs such as temperature, pulse, blood pressure, respiratory rate, pulsed oxygen saturation (SPO2), and heart rate were taken.

Respiratory functional signs such as the presence of a cough and dyspnea as well as other symptoms of COVID-19 were looked for. The complete physical examination of each patient was performed by a clinical physician

• Paraclinical parameters

Cardiac: An ECG was performed (with 12 leads) to look for a cardiac abnormality, in particular a prolongation of the QT interval (> 500 ms).

Blood culture planned for relapsing fever after symptomatic treatment.

A nasopharyngeal swab for RT-PCR was taken

The samples collected were stored at 2-8 ° C and sent to the National Laboratory of Reference Grippes (LNR-G).

Viral RNA was extracted from Oropharyngeal and / or Nasopharyngeal swabs using the QlAamp RNA Viral Kit (Qiagen, Heiden, Germany) according to the manufacturer's instructions.

Real-time RT-PCR was performed using primers and probe from ThermoFisher's TaqPath COVID-19 CE-IVD RT-PCR Kit. The ORF 1 ab, N and S genes of the 2019 novel coronavirus (2019-nCoV) are selected as target regions for amplification.

The Applied BiosystemsTM 7500 Fast Real-Time instrument was used for amplification.

A specimen is positive if it shows an obvious amplification curve with a cycle thresholds (CT) value less than or equal to 37 (CT≤ 37) for the three ORF genes l ab, N, S or for two of the genes.

The viral load of SARS-CoV-2 was assessed indirectly by the number of CT.

c. Activities of 3rd 4th 5th follow-up visit (V3, V4, D5: D7, D14, D21)

• Clinical parameters

Anthropometric parameters such as weight were measured. Vital signs such as temperature, pulse, blood pressure, respiratory rate, pulsed oxygen saturation (SPO2), and heart rate were taken.

Respiratory functional signs such as the presence of a cough and dyspnea as well as other symptoms of COVID-19 were looked for. The complete physical examination of each patient was performed by a clinical physician

• Paraclinical parameters

Cardiac: An ECG was performed (with 12 leads) to look for a cardiac abnormality, in particular a prolongation of the QT interval (> 500 ms).

Biological: complete blood count (CBC) and ESR have been performed

Biochemical: the assay of transaminases (ALAT, ASAT), serum creatinine, glycemia and C-Reactive Protein (CRP) was performed

Blood culture was planned for relapsing fever after symptomatic treatment.

A nasopharyngeal sample was taken for RT-PCR and assessment of the viral load.

2.3.8. Judging criteria

A. Primary endpoint

The rate of immediate reactions (IR) and reactions in the days after administration of the first dose: side reactions (RS)

• RI: anaphylactic shock

• RI + RS: cough (duration) diffuse erythema (duration), exanthema (duration), urticaria (duration), angioedema (duration), fatigue (duration and intensity), fever (duration and intensity), headache (duration and intensity), asthmatic attack (duration and intensity), loss of consciousness (duration in minutes), dizziness (duration and intensity), pallor (duration), sweating (duration), nausea (duration and intensity), tachycardia (duration and intensity) , diarrhea (duration and intensity), vomiting (duration and intensity), prolongation of the QT interval> 500 ms).

For any serious adverse reaction (SAE) that took place between inclusion and end of the study, the relationship between SAEs and the trial drug had to be established by the investigator, who will use the following definitions: no linked, possible link, probable link, established link.

B. Secondary endpoints:

The efficacy of the phytomedicine APIVIRINE on the viral load of COVID-19:

• Viral load clearance time based on RT-PCR CTs

• Proportion of RT-PCR negative patients on D4, D7, D14, D21

• Time to clearance of fever and clinical signs present at patient admission

The acceptability to treatment of the phytomedicine APIVIRINE:

• Proportion of participants accepting the taste

• Rate of adherence to the therapeutic regimen

2.3.9. Data managment

Data collection using the patient's notebook.

The data from the inclusion visits, the follow-up visits and the results of the various examinations were recorded in the patient's case report form (CRF), opened for each patient upon inclusion in the study. These data were subject to quality control and validation during inclusion (D1) and follow-up (D4, D7, D14 and D21) visits.

The CRF allowed the identification of the patient and the site; recording the selection and inclusion of patients in the study; the recording of all data collected at each study visit; recording of possible side effects and any exclusion from the study. All investigators had a common source document template. Each patient had their own source document file with all of the original documents, including lab results, record books, and prescriptions. These data were then entered into the CsPro software. Double entry was made to limit the number of missing data and errors in the study.

Data security

For data backup during the study, the notebooks were arranged in sets of 10, bagged and stored in a cardboard box. The storage boxes were stored in a storage cabinet in a secure room at the IRSS / Ouagadougou, the keys of which are held only by the patient monitoring team and the data manager. At the end of the study, a scanned copy of each file was made and all the storage boxes moved to a sealed storage cabinet within the IRSS enclosure.

Anonymity and data entry

To ensure data confidentiality, a unique identifier has been assigned to each patient included.

In order to make the data available at the end of the study, the data were entered gradually until the date of the end of the study. A second data entry in the IRSS premises was made and the data table used for data validation. A data entry interface designed on CsPro was used for data entry. The data cleaning after several comparisons of the data entered on site and the data entered at the IRSS / Ouagadougou was done by the biostatistician. The initial data tables and those resulting from successive comparisons were kept in addition to the final data table.

Statistical analysis

Statistical treatment was carried out on the 45 patients in the study, considering an intention-to-treat analysis. An exploratory analysis including assessment of completeness, examination of the tails of the quantitative data distribution, and identification and correction of erroneous data was first done. It was followed by an analysis of the descriptors of the sample in order to define the socio-demographic, clinical and biological profiles of the patients on inclusion. Central tendency and dispersion statistics were calculated. Depending on the distribution of values, the mean ± standard deviation or the median (1st quartile; 3rd quartile) was used to reduce the data. Likewise, frequencies were calculated to describe patient characteristics, symptoms and clinical signs. The

proportions of patients exhibiting clinical symptoms and signs of the disease during follow-up visits were calculated as well as the proportions of patients exhibiting adverse reactions.

In addition, all serious side effects that occurred during the study had to be described in detail.

Statistical tests and regression analysis

The upper one-sided z-test was used to assess the null hypothesis. Discrete-time survival methods, in particular log-rank test / Haenszel type methods, were used to estimate the time before virus elimination by analyzing repeated evaluations of viral carriage negativation. A significance level of 5% was considered statistically significant in all analyzes. All analyzes were performed using stata 14.1 software and R software version 3.6.3.

Analysis of tolerance parameters

Descriptive analysis was used to assess the safety of the study drugs.

The safety and tolerance of the drug were evaluated on the basis of clinical parameters and laboratory tests. Clinical signs and symptoms were compared by incidence, intensity, severity, and relationship to study drugs. Likewise, laboratory parameters including results of hematology and biochemistry tests were analyzed against normal values to determine the level of safety of the product. Safety profiles for all parameters were analyzed at each time point to determine clinical and laboratory changes from baseline.

2.3.10. Ethical and deontological aspects

The protocol received a favorable opinion after examination by the national ethics committee for health research of the Ministry of Health.

The study investigators explained the protocol to the participants in plain language. The participants were informed about the advantages and disadvantages. Trial insurance was purchased as part of the study.

An informed consent form was signed by the participants after all the information and explanations received on the study and which will attest to their agreement to take part in the study. Non-consenting patients were treated according to the national protocol in force at the time of the study (hydroxychloroquine-based protocol).

The digital borrowing has been applied for the illiterate. Confidentiality of patient information was ensured throughout the study. Participants were free to withdraw their consent at any time in case of doubt.

Clinical monitoring was provided by clinical physicians who were trained in good clinical practices, the study protocol and the collection of informed consent as well as filling in the observation booklet. The study participants were fully supported by the study, as well as the additional exams.

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Health Research Ethics Committee (CERS) of Burkina Faso (N ° 2020.7.121).

3. RESULTS / INTERPRETATIONS

3.1. Patient characteristics

3.1.1. Socio-demographic characteristics

- **Age and sex**

The present study included 45 patients. The female sex was represented by 53.3% or 24 patients. The median age was 31 years (27 years; 46 years) with extremes of 20 years and 64 years. The distribution of patients by gender and age is presented in Table I.

Table I: Distribution of patients by gender and age

Variables	Workforce (n)	Percent (n / N)
Kind		
Male	21	46,7
Female	24	53,3
Total	**45**	**100**
Age category		
< 30	16	35,6
30-49	23	51,1
50-65	06	13,3
Total	**45**	**100**

- **The residence**

The distribution of patients by residence showed that they came from 09 districts of the city of Ouagadougou. Their number varied from 02 to 10 patients and those from district 11 were the largest. This distribution is presented in Table II.

Table II: Distribution of patients by residence

District t	Staff (n)	Percentage (%)
02	08	17,78
03	02	04,44
04	06	13,34
05	04	08,89
06	02	04,44
07	02	04,44
10	08	17,78
11	10	22,22
12	03	06,67
Total	**45**	**100**

3.1.2. Clinical characteristics of patients at inclusion

- **Circumstances of discovery**

Contact cases were the most represented with 22 patients. Table III summarizes the circumstances of discovery of COVID-19 in our patients.

Table III : Circumstances of discovery

Reasons for consultation	Staff
Contact case	22
Travel	12
Functional and general signs	11
Total	**45**

- **Clinical signs**

The clinical signs present at inclusion were mostly cough (20 patients), followed by asthenia (19 patients), headache (18 patients) and anosmia (16 patients). Dyspnea and chest pain were poorly represented in 05 and 06 study patients, respectively.

Note that the same patient could present several clinical signs on inclusion. The representation of patients according to clinical signs at inclusion is shown in Table IV.

Table IV: Representation of patients according to clinical signs at inclusion

Reasons for consultation	**Staff (n)**
Cough	20
Asthenia	19
Headache	18
Anosmia	16
Fever	12
Nasal obstruction	10
Sore throat	10
Chest pain	06
Dyspnea	05

3.2. Paraclinical characteristics

3.2.1. Biological characteristics

One (01) patient (2.22%) had AS hemoglobinopathy. Blood group A with rhesus positive was found in 21 (46.67%) patients. C-reactive protein (CRP) and sedimentation rate (ESR) markers of inflammation were elevated in 15 (33.33%) and 24 (53.33) patients, respectively. Three (03) patients had elevated serum creatinine. As for transaminases, ALTs were elevated in 05 (11.11%) patients, while ASATs were elevated in 04 (8.89%) patients.

The distribution of patients by biological characteristics is shown in Table V.

Table V: Distribution of patients according to the biological characteristics at inclusion.

Biological assessment	Number (n = 45)	Percentage (%)
Electrophoresis		
AA	44	97,78
AS	01	02,22
Total	45	**100**
Blood group		
A+	21	46,67

B+	15	33,33
0+	07	15,56
0-	02	04,44
Total	**45**	**100**
High CRP	15	33,33
VS high	24	53,33
Abnormal leukocytes	13	28,89
PNN abnormal	11	24,44
Abnormal NLP	12	26,67
Abnormal hemoglobin level	29	64,44
Elevated platelets	03	06,67
ALAT high	05	11,11
AST high	04	08,89
Elevated creatinine	03	06,67
Elevated blood sugar	11	24,44

3.2.2. CT features

Thoracic CT was performed in all patients at baseline. Abnormal polished glass images were found in 10 (22,22) patients.

The lesions found were of the interstitial and / or alveolar type. The distribution of patients according to CT characteristics is shown in Table VI.

Table VI: Distribution of patients according to CT characteristics

CT	Numbers (n)	Percentage (%)
Unnatural	10	22.22
Type of anomaly		
Polished glass picture	10	100
Alveolar opacity		
Unilateral	02	20
Bilateral	08	80
Total	**10**	**100**

Interstitial opacity		
Unilateral	03	33,33
Bilateral	06	66,67
Total	**09**	**100**

3.3. Assessment of the tolerance and safety of APIVIRINE

3.3.1 Acceptability

During the treatment, 03 patients missed hours of taking the drug respectively of one dose for 01 patient and three doses for 02 patients. The taste of the drug was not the basis for the lack of intake, on the other hand forgetting was the only reason for this non-compliance.

The acceptability of odor and dosage has not been evaluated. However, some patients have reported finding the treatment regimen restrictive due to the high number of doses per day.

3.3.2 Characteristics of adverse effects during the study

Clinical monitoring

During this study, no serious adverse effects were reported. However, a case of diarrhea was recorded in one (01) patient who declared to have consumed pork, yogurt. This diarrhea stopped after taking an anti-diarrhea (loperamide tablet: 2 mg).

The evolution of symptoms during treatment is shown in Table VII.

Table VII: Evolution of symptoms during treatment

Effects undesirable	**Visitl = Inclusion (n = 45)**	**Visit2 (n = 45)**	**Visit3 (n = 30)**	**Visit4 (n = 20)**	**Visit5 (n = 3)**
Paresthesia *	0 (0)	1 (2,22)	0 (0)	0 (0)	0 (0)
Respiratory distress	1 (2,22)	0 (0)	0 (0)	0 (0)	0 (0)
Dyspnee	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nausea	2 (4,44)	2 (4,44)	1 (3,33)	0 (0)	0 (0)
Diarrhea *	0 (0)	0 (0)	1 (3,33)	0 (0)	0 (0)

Vomiting	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Abdominal pain	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pruritus	2 (4,44)	0 (0)	0 (0)	0 (0)	0 (0)
Rash	**1** (2,22)	1 (2,22)	0 (0)	0 (0)	0 (0)
Bone pain	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Infammations members	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other signs	Constipation (01) Dizziness (02)	Anosmia ageusia (01)	None None	None None	None None

EKG monitoring

The electrocardiogram was performed in all patients (100%) at inclusion, then during the visits. No abnormal heart rhythms were observed in any of the patients in the study.

3.3.3 Evolving biological characteristics of the patients included in the study

Biological data were collected during visits V1 (inclusion), V3, V4 and V5. The results showed that at the inclusion visit, CRP, leukocytes and glycemia were abnormal respectively in 15 (33.33%), 13 (28.89%), 11 (24.44%) patients. At visit 5, these parameters improved gradually in the patients who remained in study treatment. The evolution of the biological data during the various medical visits is presented in Table VII.

Table VII: Evolution of biological data during the various medical visits

Biological assessment	**Inclusion (n = 45)**	**Visit2**	**Visit3 (n=26)**	**Visit 4 (n=16)**	**Visit 5 (n=1)**
Abnormal CRP	15 (33,33)	07 (26,92)		01 (6,25)	00 (0
VS abnormal	24 (53,33)	14 (53,85)		02 (12,50)	01 (100)
Leukocytes **Unnatural**	13 (28,89)	04 (15,38)		00 (0,00)	00 (0)
PNN abnormal	11 (24,44)	03 (11,54)		01 (6,25)	00 (0)

PNL abnormal	12 (26,67)	08 (30,77)		02 (12,50)	00 (0)
PNL abnormal					
Hemoglobin level abnormal	29 (64,44)	-	13 (50)	07 (43,75)	01 (100)
Abnormal platelets	03 (6,67)	00 (0)		00 (0)	00 (0)
Abnormal ALAT					
Men	02	01		00	00 (0)
Women	03	01		01	00 (0)
Total	05 (11,11)	02 (7,69)		01 (6,25)	00 (0)
ASAT abnormal					**00** (0)
Men	**01**	00		00	00 (0)
Women	03	01		00	00 (0)
Total	04 (8,89)	01 (3,85)		01 (6,25)	00 (0)
Abnormal creatinine	03 (6,67)	01 (3,85)		00 (0)	00 (0)
Men	03	00		00	00 (0)
Women	03	**01**		**00**	**00** (0)
Total	03 (6,67)	-	01 (3,85)	00 (0)	00 (0)
Abnormal blood sugar	11 (24,44)	-			

3.4. Efficacy of APIVIRINE

3.4.1. Clinical efficacy

3.4.1.1. Constants

During the study, constants were measured in all patients during each medical visit. The temperature was measured three times during the same visit and at different times (on arrival TI, during visit T2, at the end of visit T3). Median temperature values were normal at all medical visits.

Abnormal (high) maximum temperature values were recorded during the medical examination V1 at times T1 = 38.7 ° C; T2 = 38.9 ° C; T3 = 39.2 ° C. These maximum data were normalized during the V2, V3, V4 and V5 medical visits.

The changes in the constants during processing are shown in Table VIII.

Table VIII: Variation of constants during treatment

constants	**Inclusion (n=45)**	**Visit2 (n=45)**	**Visit3 (n=30)**	**Visit4 (n=20)**	**Visit5 (n=3)**
Temperature **1**	**37,2**(35,9;38,7)	**37**(36;37,8)	**36,9**(36,1;37,9)	**37**(36,5; 37,7)	**36,7**(36,7;37,6)
Temperature 2	**37,1**(35,9; 38,9)	**37**(36,1;37,8)	**36,8**(36,1;37,8)	**37**(36,5;37,6)	**36,7**(36,7;37,7)
Temperature 3	**37,1**(35,8; 39,2)	**37**(36,1;37,8)	**36,9**(36,1;37,8)	**36,9**(36,5;37,7)	**36,8**(36,6;37,6)
Respiratory rate	**19**(16 ; 30)	**18** (17 ; 23)	**18** (16 ; 22)	**18,5** (18 ; 19)	**18** (18; 20)
Saturation (SpO2)	**98** (94 ; 100)				
Pulse	**81** (52 ; 118)	**84** (52 ; 117)	**84,5** (52 ; 101)	**84,5** (53 ; 89)	**86** (67 ; 88)
Systolic blood pressure	**127 (100 ;** 178)	128(100;175)	128 (113 ; 167)	**128,5** (120; 57)	**125** (125;137)
Voltage diastolic	**77** (63 ; 109)	**76,5** (67 ; 97)	**77** (67 ; 97)	**79** (69 ; 92)	**76** (69 ; 82)

3.4.1.2. Symptoms

Signs were collected on inclusion of patients and were monitored throughout treatment (Table IX). Certain signs were associated or not in the same patient. Thus, major symptoms such as cough, asthenia, headache and anosmia were found in 20, 19, 18 and 16 patients, respectively.

These signs progressed to disappearance at the second visit with the exception of anosmia and asthenia which disappeared at visit 3 in 1 and 2 patients, respectively. The evolution of functional signs during treatment are recorded in Table IX.

Table IX: Frequency of functional signs during treatment

Symptoms	**Inclusion (n=45)**	**Visit2 (n=45)**	**Visit3 (n=30)**	**Visit4 (n=20)**	**Visit 5 (n=3)**
Cough	**20**	**00**	**00**	**00**	**00**
Dyspnea	**05**	**00**	**00**	**00**	**00**
Thoracic pain	**06**	**00**	**00**	**00**	**00**
Anosmia	**16**	**1**	**00**	**00**	**00**
Nasal obstruction	**10**	**00**	**00**	**00**	**00**
Asthenia	**19**	**2**	**00**	**00**	**00**
Fever	**12**	**00**	**00**	**00**	**00**
Headache	**18**	**00**	**00**	**00**	**00**
Sore throat	**10**	**00**	**00**	**00**	**00**

Concomitant treatments

During the study, some 06 patients (19.35%) had concomitant treatments. These treatments consisted mainly of: analgesic and antipyretic (paracetamol), vitamin C, antihistamine (loratadine), steroidal anti-inflammatory drug (betamethasone), antibiotic (amoxicillin + clavulanic acid), antidiarrhoeal (loperamide)), anti-anemic (iron), progesterone, zinc and anti-cough phytomedicine (DOUBA syrup). Table X shows the distribution of concomitant treatments in the patients.

Table X : Breakdown of concomitant treatments

Pharmacological class	Effective (n)
Antihistamine	02
Anti inflammatory steroid	01
Progestogen	01
Analgesic / antipyretic	02
Anti cough	01
Antibiotic	01
Anti anemic	01
Vitamin	01

3.4.1.3. Examination of devices and systems

The examination at inclusion revealed pulmonary abnormalities in 03 patients. Abnormalities of the skin and appendages (macules, depigmentation) were found in 03 other patients unrelated to COVID-19. Only the lung abnormalities were attributable to COVID 19 and improved at the V3 visit. The anomalies noted during the examination of the devices and systems are presented in table XI.

Table XI: Anomalies in the examination of devices and systems

Device examination	**Inclusion (n = 45)**	**Visit2 (n =45)**	**Visit 3 (n = 30)**	**Visit 4 (n=20)**	**Visit5 (n= 3)**
Status of abnormal consciousness	**00** (0)	**00** (0)	**00** (0)	**00** (0)	**00** (0)
Conjunctiva abnormal	**00** (0)	**00** (0)	**00** (0)	**00** (0)	**00** (0)
Joint abnormal	**00** (0)	**00** (0)	**00** (0)	**00** (0)	**00** (0)
Pulmonary abnormal	**03** (6,67)	**01** (2,22)	**00** (0)	**00** (0)	**00** (0)
Cardiovascular abnormal area	**00** (0)	**00** (0)	**00** (0)	**00** (0)	**00** (0)
Digestive abnormal	**00** (0)	**00** (0)	**00** (0)	**00** (0)	**00** (0)
abnormal skin integuments	**03** (6,67)	**02** (4,44)	**01** (3,33)	**01** (5)	**00** (0)
Details on abnormal skin and integuments	Dépigmentation (1) Macule (2)	Dépigmentation (1)	Dépigmentation (1) Macule (1)	Dépigmentation (1)	

3.4.2. Virological cure

3.4.2.1. RT-PCR

RT-PCR was performed in all patients during the second visit, as well as at the third, fourth and fifth scheduled visits when the result of the previous visit was still positive.

After 04 days of treatment, the cumulative cure rate was 33.33% and 48.89% after 7 days. The cumulative cure rate was 86.67% after 14 days of treatment. On the 15th day of treatment, this cumulative cure rate was 93.33%.

The representation of virological cures is given in Table XI.

Table XI: Representation of virological cures

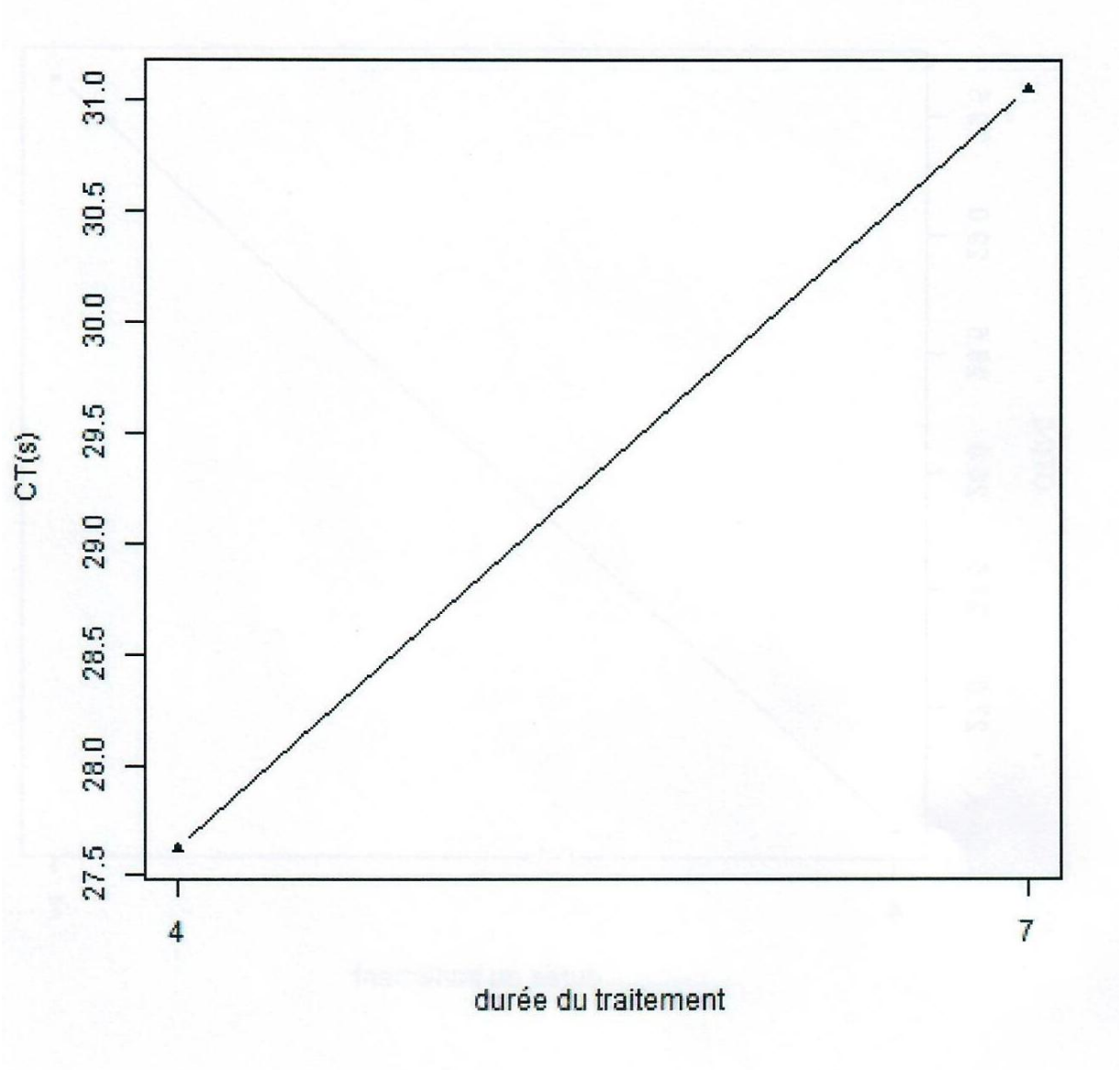
Duration of treatment before cure (days**)**	**Workforce cumulative**	Cumulative percentage (%)
04	15	33,33
07	22	48,89
14	**39**	**86,67**
15	**42**	**93,33**
21	45	100,00

3.4.2.2. Viral load

Virological monitoring by RT-PCR consisted of looking for three genes specific to SARS CoV2 (N, S, ofrl ab). The identification of each gene was made by the number of Cycles Thresholds (CT). The change in viral load is inversely proportional to the number of CTs.

The results show a clear increase in the cycles of replication of the 3 genes between D4 and D7 (48.89% of patients) (figure 2, 3, 4). This increase reflects a drop in viral load (up to negation) for the 3 genes detected by the platform.

Between D7 and D14, the results objectify a maintenance of the increase in CT for the S and O genes. For the N genes, despite a decrease in CT, the kinetics remain significantly higher than the initial CTs (figure 5, 6, 7).



Duration of treatment

Figure 2: progression curve of CT(S) up to seven days of treatment

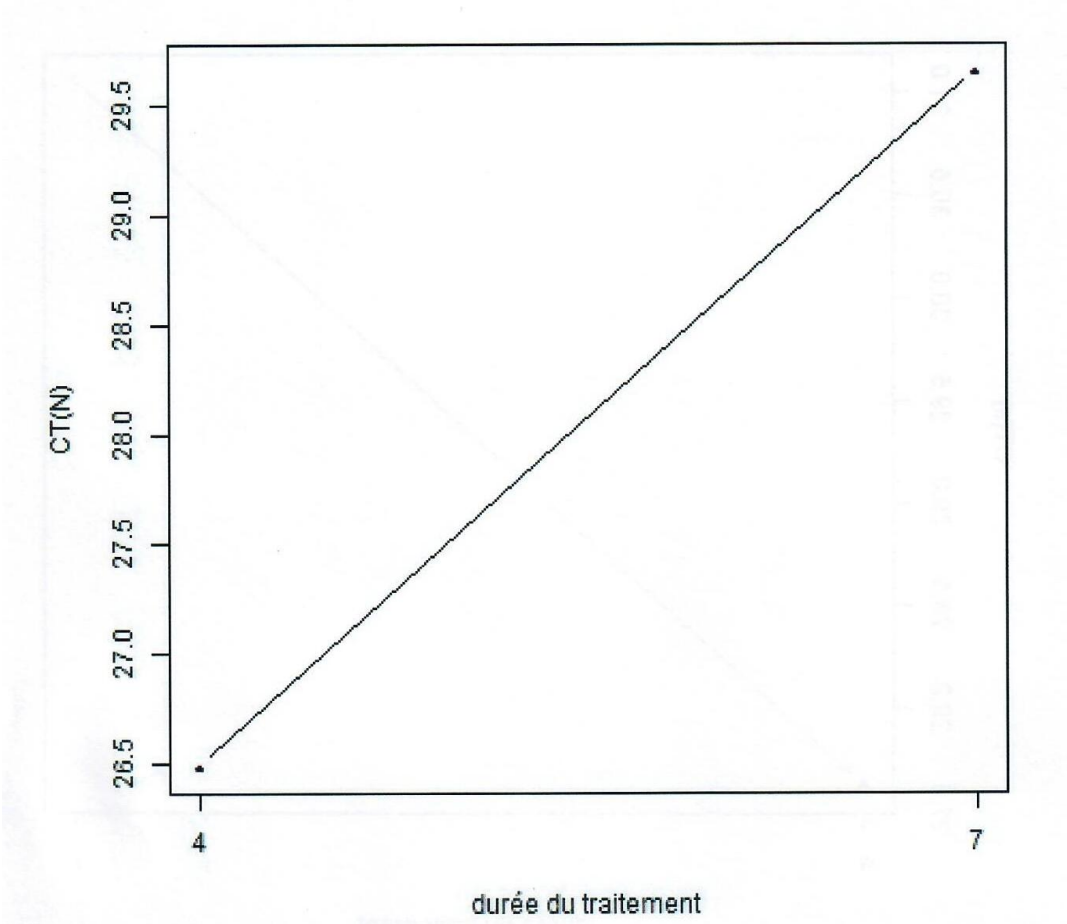


Figure 3:courbe évolutive de la CT(N) jusqu'à sept jours de traitement

Duration of treatment

Figure 3: Progression curve of CT (N) up to seven days of treatment

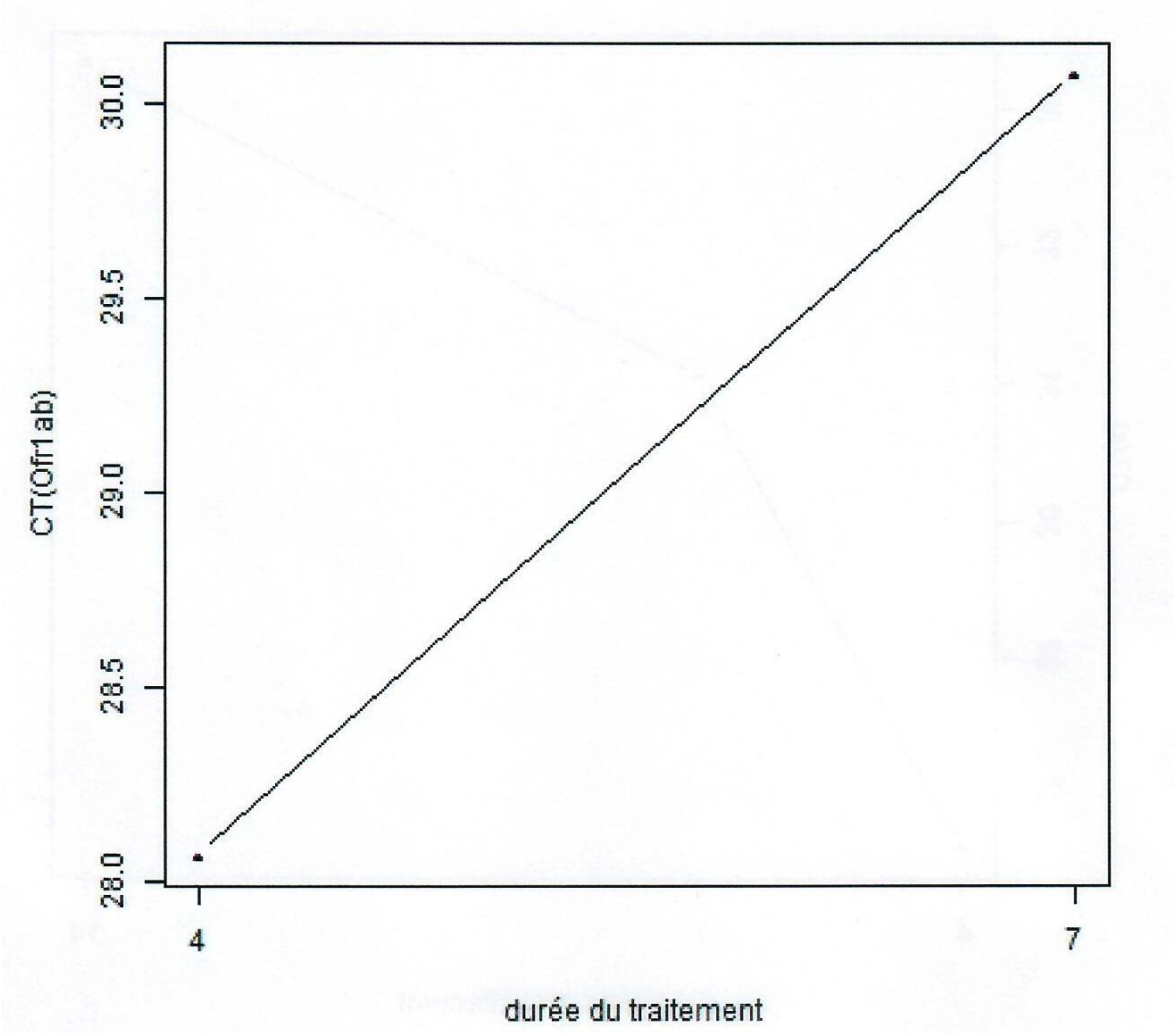


Figure 4:courbe évolutive de la CT (ofr ab) jusqu'à sept jours de traitement

Duration of treatment

Figure 4: Progression curve of CT (ofr ab) up to seven days of treatment

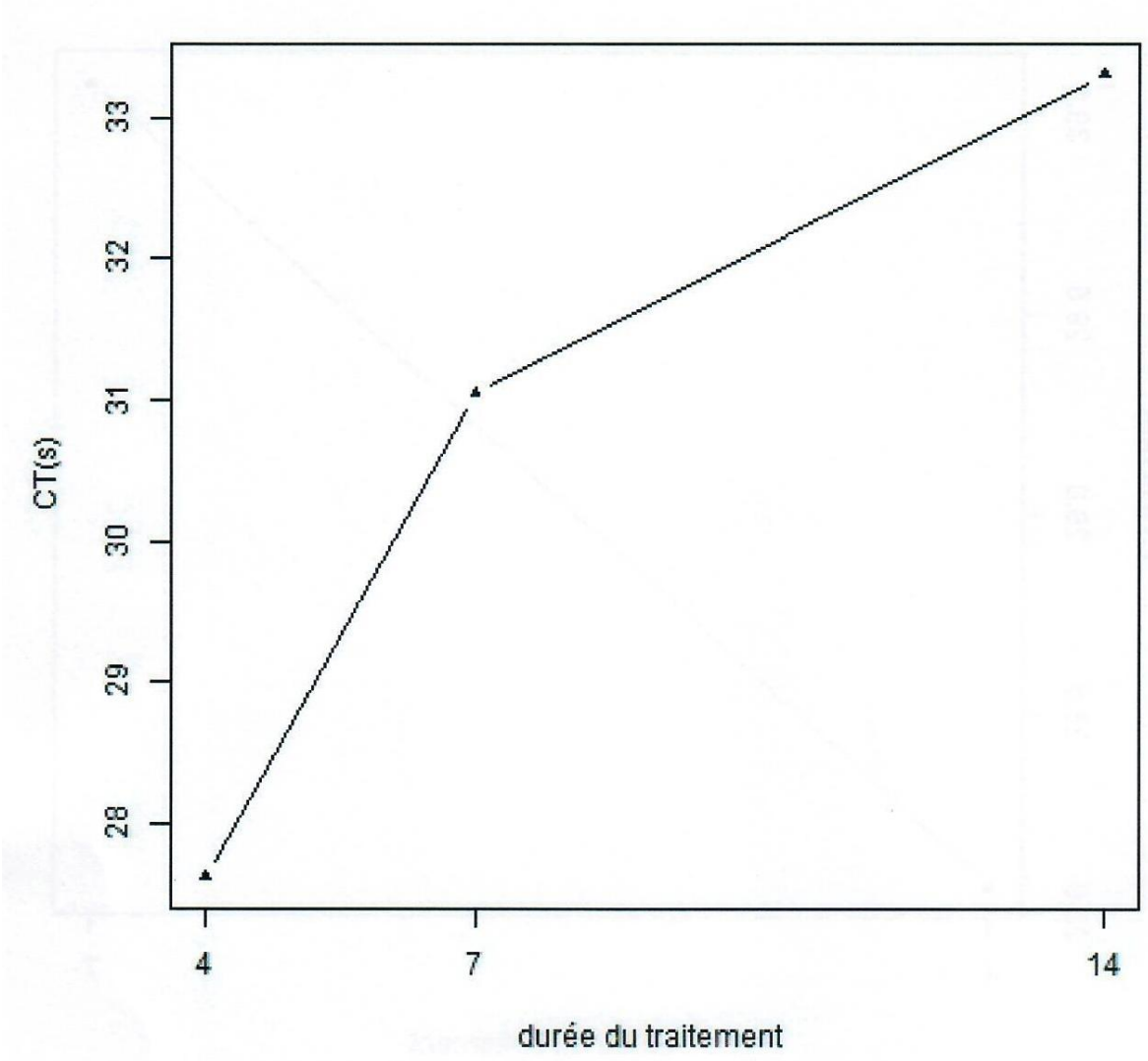


Figure 5: courbe évolutive de la CT(S) jusqu'à quatorze jours de traitement

Duration of treatment

Figure 5: Progression curve of CT (S) up to fourteen days of treatment

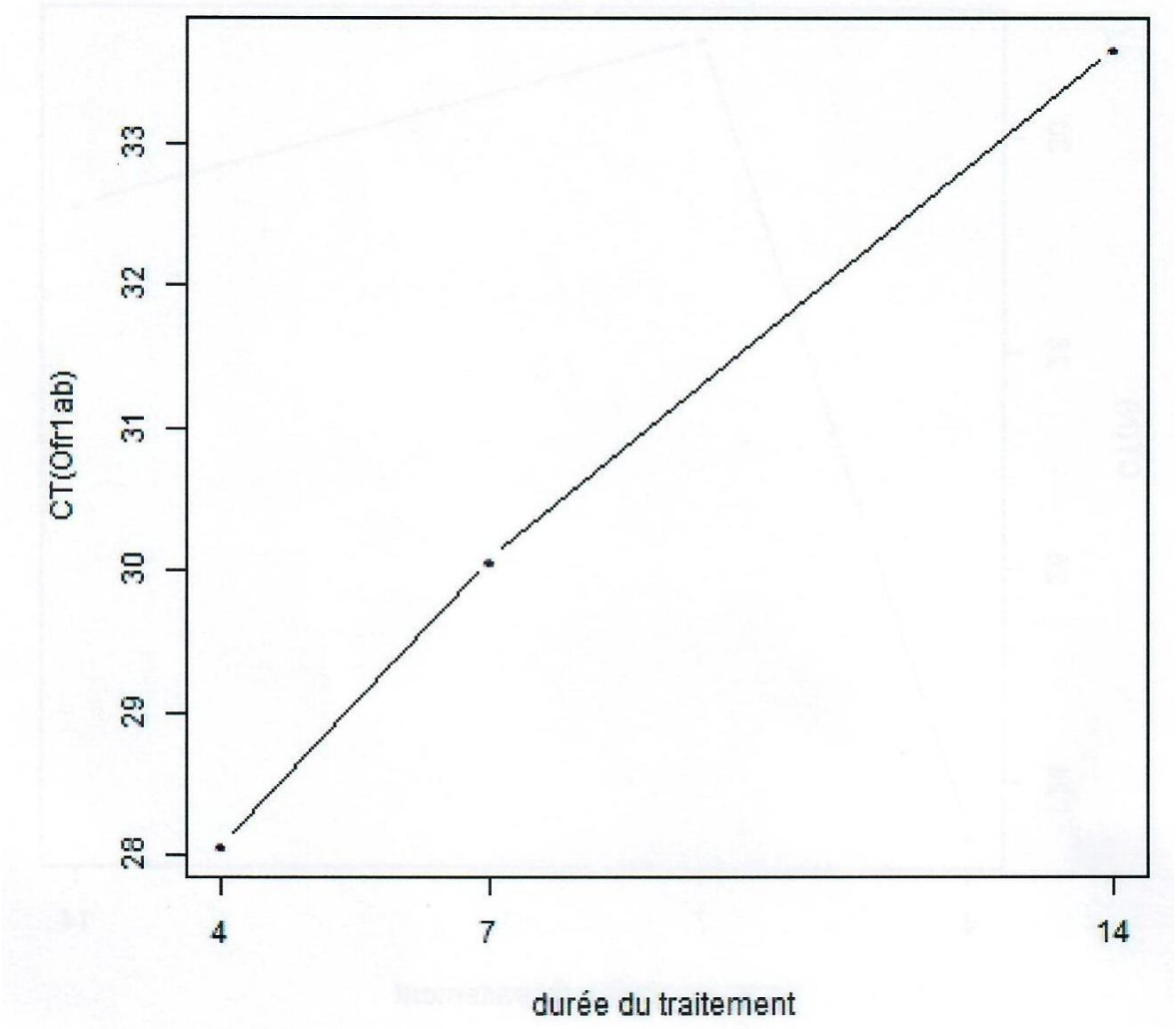


Figure 6:courbe évolutive de la CT (ofr 1 ab) jusqu'à quatorze jours de traitement

Duration of treatment

Figure 6: Progression curve of CT (ofr 1 ab) up to fourteen days of treatment

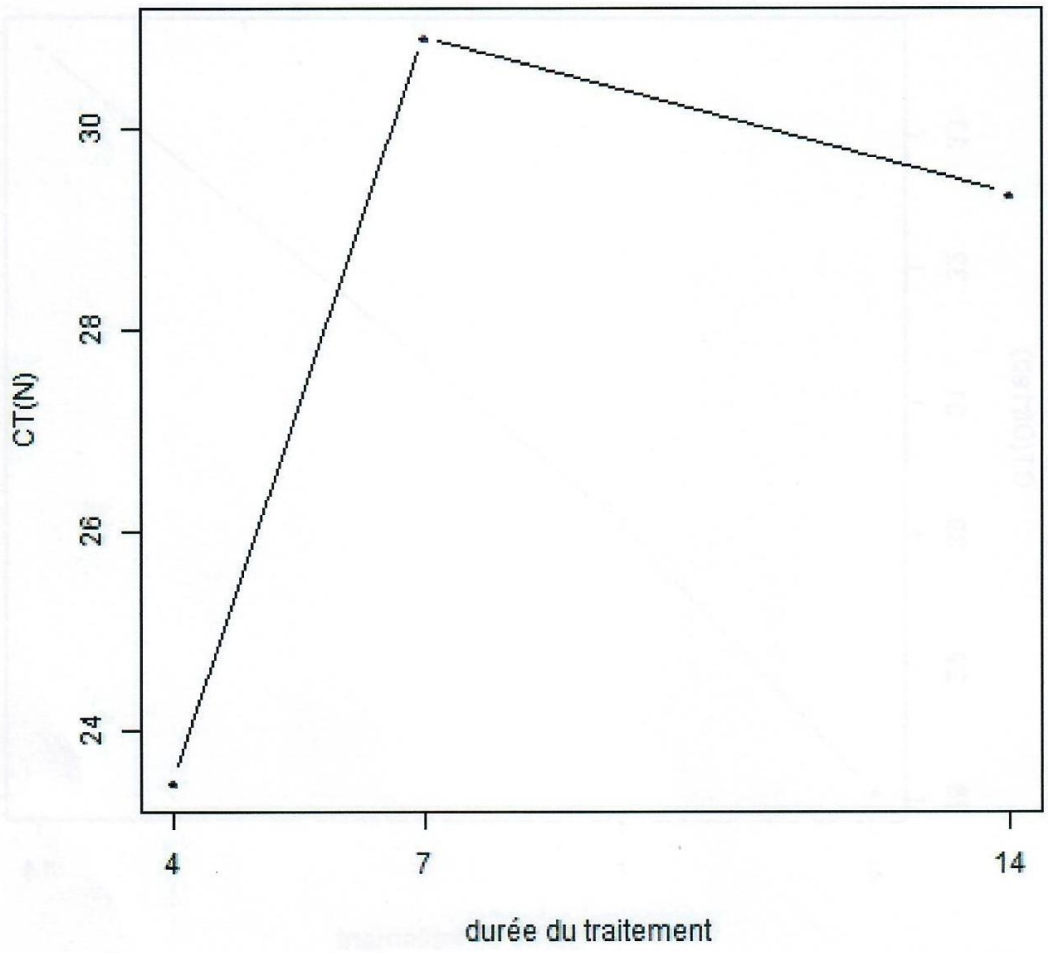


Figure 7: courbe évolutive de la CT(N) jusqu'à quatorze jours de traitement

Duration of treatment

Figure 7: progression curve of CT(N) up to fourteen days of treatment

4. Monitoring and quality assurance

A first monitoring visit consisted of an evaluation of the paramedical examinations laboratories. The main purpose of the second monitoring visit on October 30, 2020 was to review the system put in place for the start of the activities of the APIVIRINE clinical trial. The meeting focused on the operationalization of the field team and the various. Two weeks after the effective start of the clinical trial, on November 11, 2020, a meeting was held at the end of the third monitoring visit to analyze the difficulties encountered in the field and the monitoring of probable adverse effects. Non-conformities in the regularity of meeting appointments and the effectiveness of initial assessments were addressed. Most of the difficulties related to the operation of the field team noted during the start-up monitoring meeting of October 30, 2020 have been resolved, namely the performance of examinations such as the ECG, the chest scanner and the availability of a vehicle. . However, others only found alternative solutions (consumables and media). The difficulties encountered, among other things, travel during non-working hours of the public service, communication credits, psychological support and the need for an interview guide have found concrete proposals made by the principal investigator. It was proposed to take measures to make vehicles available 24 hours a day for the movement of doctors in the event of an emergency. To ensure the follow-up of patients, it has been proposed to make telecommunications funds available to investigators. Four weeks after the effective start of the clinical trial (November 27, 2021), a fourth monitoring visit had the general objective of taking stock of the progress of activities. A check of the completeness of the data in the observation notebooks followed by discussions on the progress of the study and the difficulties as well as proposals for improvement solutions. A fifth and final monitoring visit on December 21, 2020 which consisted of taking stock of the current state of the patients included and also of the difficulties. At the end of this visit a proposal to the principal investigator to make a partial analysis of the raw data on the patients of the study was made. Then took place the transfer of the observation notebooks to the research institute in health science for the entry and analysis of study data.

5. Limits

The limitations of this study can be summarized as follows:

- The change in the national protocol for the management of positive COVID-19 cases did not allow the follow-up in a hospital environment of patients without signs of seriousness;

- The study was carried out on an outpatient basis and medication intake could not be directly observed except during scheduled visits.

6. CONCLUSION

The results of the clinical trial on a sample of 45 patients showed good tolerance of the phytomedicine in all of the patients in the study. No immediate reaction was observed. Renal and hepatic function were not affected in the treated patients. The electrocardiogram also did not reveal any disturbance of the heart rhythm during treatment. Also, the patients did not have serious side effects requiring management during treatment.

Patients with disease symptoms at baseline experienced amendment within 4 days of starting treatment. All symptoms disappeared after 07 days of treatment. In addition, patients who had an abnormality in serum creatinine and transaminases at baseline experienced a normalization of these parameters during treatment.

The cure rate after 14 days of treatment was 86% versus an 80% threshold targeted at the start of the study. However, the cure rate was 93.33% on day 15. In addition, the patients who were not negative at the first and second check-ups experienced a significant drop in viral load. These results are in favor of an efficacy of the phytomedicine on the replication of the COVID-19 virus.

It would be wise to undertake subsequent preclinical experiments which would make it possible to establish the molecule or molecules at the origin of the inhibitory effects of viral replication and their mechanism of action.

From the point of view of acceptability, the herbal medicine and the treatment regimen were well accepted by the patients.

In short, through this study the harmlessness and safety of the phytomedicine could be demonstrated in view of the absence of adverse effects but also the acceptability of the therapeutic protocol.

7. Outlook

Thus, the phase II clinical trial (API-COVID-19) yielded satisfactory results. The outlook would confirm the efficacy of APIVIRINE on:

- A preclinical study on viral and anti-inflammatory activities in vitro and their mechanisms of action,
- A multicenter phase II / III clinical trial on a large sample,
- A request for Marketing Authorization.

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Ouagadougou, September 15, 2021

Ouagadougou, le 15 septembre 2021

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