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A *CRYPTOLEPIS SANGUINOLENTA*-BASED PREPARATION REDUCED HOSPITAL STAY AND MORTALITY OF COVID-19 PATIENTS DURING THE PANDEMIC

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Abstract

Background: In 2020, the World Health Organization urged nations to take domestic steps to curb the spread and contain the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic, also referred to as coronavirus disease 2019 (COVID-19). In the meantime, there was growing clinical evidence that reported favourable effects of using herbal medicine in managing COVID-19. We, therefore, repurposed Nibima (a *Cryptolepis sanguinolenta*-based herbal preparation) as an adjunct to conventional therapy for COVID-19. This was premised on our previous *in vitro* investigations that showed that Nibima has both antiviral and anti-inflammatory activities.
Materials and Methods: This trial (FDA/CT/11) was an open-label, randomised controlled phase II pilot study, which was conducted from March 2021 to October 2022 among hospitalised COVID-19 patients in the Ashanti Region of Ghana. Participants were either randomised to aqua (water) or Nibima, the investigational product as adjunct to COVID therapy.
Results: Nibima was well tolerated by the study participants, and the patients who were administered that together with the standard COVID-19 therapy had rapid clinical recovery compared to those taking aqua and the standard or conventional COVID-19 therapy. The evidence obtained provides a strong basis for future studies with large numbers of hospitalised patients with COVID-19 and other viral respiratory infections.

Conclusion: Though COVID-19 is no longer a pandemic, it is still a public health challenge. We recommend that Nibima be further investigated for its broad-spectrum antiviral and immunomodulatory activities against SARS-COV-2 and other viral infections in preparedness for the re-emergence of future pandemics.

Keywords: *Cryptolepis sanguinolenta*; COVID-19, interferon induction; phase II clinical trial

List of Abbreviations: COVID-19; Coronavirus Disease 2019; CPMR; Centre for Plant Medicine Research FDA; Food and Drugs Authority; IP; investigational product; LMICs; Low- and Middle-Income Countries PIN: Personal Identification Number; RNA; Ribonucleic Acid; SARS-COV-2 Severe Acute Respiratory Syndrome Coronavirus 2; WHO; World Health Organisation

Introduction

Three years after the coronavirus disease 2019 (COVID-19) pandemic started in China's Yuhan Province in 2019, it is still raging on, with some affected patients developing long-term COVID-19 complications. According to the WHO, as of December 2023, over 772 million confirmed cases and nearly seven million people have died from COVID-19 and related complications. SARS-CoV-2 is the virus responsible for COVID-19, which can induce a wide range of clinical manifestations, such as an infection with no symptoms to a potentially fatal condition (Kiem *et al.,* 2020). The SARS-CoV-2 is an enveloped positive-sense RNA virus belonging to the *Coronaviridae* family, the *Orthocoronavirinae* subfamily, the *Betacoronavirus* genus, and the *Sarbecovirus* subgenus (Chen *et al.,* 2020; Guan *et al.,* 2020). More severe disease is characterised by lower respiratory tract infection, pneumonia, and respiratory failure, which results in death in about 0.5% of confirmed cases (Kiem *et al.,* 2020). Before the SARS-CoV-2 outbreak in 2019, two outbreaks of coronaviruses (SARS-CoV in 2002 and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in 2012) emerged and caused serious illnesses in the previous two decades (Chen *et al.,* 2020).

When the pandemic began in early 2020, the WHO urged nations to take domestic steps to stop the spread of the virus. Since then, numerous medical professionals and academics have worked hard to develop local-based remedies to treat COVID-19 patients and stop its spread. As a result, over 300 medications have been studied for clinical trials in various locations across the globe (Tobaiqy *et al.,* 2020; Heustess *et al.,* 2021). Also, at the start of the pandemic, there was growing clinical evidence that reported favourable effects of using herbal medicine to treat or manage COVID-19 (Chan *et al.,* 2020). The only antiviral medication used during the pandemic in evidence-based therapy was remdesivir (Beigel *et al.,* 2020), which had been demonstrated to improve hospital discharge rates, and dexamethasone (Chappell *et al.,* 2020), a broad-spectrum anti-inflammatory medication that helped patients who needed respiratory support. However, many people in low- and middle-income countries (LMICs) could not afford these medications. Hence, there was an urgent need to search for cheaper, effective anti-COVID-19 drugs locally.

All viral infections rely on the infected host's innate immune responses to limit the severity of sickness after infection starts, particularly those caused by novel strains with little to no established adaptive immunity to the pathogen. Interferons, essential orchestrators of the antiviral immune response with both powerful antiviral and immunomodulatory properties, are crucial to this natural response (Li *et al.,* 2018). One of the first cytokines produced by a viral infection of a cell is the type I interferon (interferon-α/β), which is the primary inducer of innate immune responses in the human lung (Watson *et al.,* 2020). Interestingly, coronaviruses are known to express multiple interferon antagonists for optimal inhibition of interferon signalling (Schroeder *et al.,* 2021). For instance, during the early stages of infection, SARS-CoV-2 successfully inhibits interferon production, resulting in the dysregulation of the signalling of interferons, which plays a role in exaggerated inflammation and severe lung immunopathology (Channappanavar *et al.,* 2016). Additionally, a clinical investigation of COVID-19 patients revealed that those with more severe disease had much lower interferon activity (Hadjadj *et al.,* 2020). Less interferon is produced by at-risk populations, including those with comorbidities, the elderly, and those on immunosuppressive drugs, which raises the possibility of developing more severe lung disease (Agrawal, 2013; Singanayagam *et al.,* 2019).

In 2020, during the spread of the COVID-19 pandemic, our research group showed that *Cryptolepis sanguinolenta* had broad-spectrum antiviral and anti-inflammatory activities; these data were later published (Borquaye *et al.,* 2020; Mensah-Kane *et al.,* 2020; Domfeh *et al.,* 2021a; Domfeh *et al.,* 2021b; Domfeh *et al.,* 2022). The West African medicinal plant *C. sanguinolenta* (Lindl.) Schlechter (Apocynaceae) has been reported to exhibit a plethora of pharmaco-effects, including anti-malarial, anti-hyperglycaemic, antibacterial, and anti-inflammatory effects in diverse animal models (reviewed in Osafo *et al.,* 2017). Nibima is the local name of the aqueous extract of *C. sanguinolenta* manufactured by the Centre for Plant Medicine Research (CPMR) in Mampong Akuapem, in the Eastern Region of Ghana.

Ghana's FDA has since 2010 approved Nibima (FDB/HD.07-7096) for treating uncomplicated malaria. The product is administered orally, well tolerated, and no known serious adverse reactions have been reported. The reported clinical data for Nibima as a broad-spectrum antiviral and anti-inflammatory agent (Domfeh *et al.,* 2021a; Domfeh *et al.,* 2021b; Domfeh *et al.,* 2022), coupled with its availability on the market for malaria therapy, provided the rationale for a repurposed randomised controlled, open labelled phase II trial to determine whether Nibima has the potential to reduce the severity of lower respiratory tract illness and accelerate recovery in patients diagnosed with COVID-19. We designed this clinical trial guided by the WHO R and D Blueprint Novel Coronavirus COVID-19 Therapeutic Trial

Synopsis 16 issued in February 2020 with some minor modifications [https://www.who.int/publications-detail-redirect/covid-19-therapeutic-trial-synopsis].

Materials and Methods

Study Design and Sites

This was an open-label, randomised controlled phase II trial that commenced from March 16, 2021 to October 10, 2022. Although the trial was approved for two sites in the Kumasi Metropolis of Ghana, i.e., the Kumasi South Hospital and the Komfo Anokye Teaching Hospital, yet only the Kumasi South Hospital was used. This is because upon receipt of the FDA's approval, the infection rate had significantly slowed, and most of the new cases were referred to Kumasi South Hospital as a designated site for screening, admissions, and referrals when necessary. Patients' samples were shipped to research and diagnostic laboratories at the main KNUST campus and Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) for analysis. The trial followed the approved protocol and all the laws and regulations for undertaking clinical trials, including the International Council for Harmonisation (ICH E6(R2) guidelines and Good Clinical Practice. Also, the standards set out by the Research Governance Framework and ethical principles that have their origins in the Helsinki Declaration for clinical trials were followed.

Study participants

Eligibility criteria for inclusion were adults with laboratory-confirmed SARS-CoV-2 infection (nasopharyngeal swab positive for SARS-CoV-2 by polymerase chain reaction), admitted to a health care facility for the treatment of COVID-19 complications, patients with moderate to severe COVID-19, or patients who are at high risk of progression to severe or life-threatening disease, ability to take oral medication and be willing to adhere to the study intervention regimen, willingness to comply with all study procedures and availability for the duration of the study. Informed consent provided by the patient was obtained before enrolment. None of the patients had been vaccinated for COVID-19 during the study period.

Patients younger than 18 years, contraindications to components of the investigational product (Nibima), females with pregnancy, lactation and breastfeeding mothers, and patients on treatment with herbal products in the previous 4 weeks, smokers, mentally unstable, presence of specific devices (e.g., cardiac pacemaker) were excluded from the study.

The baseline characteristics of the patients, including their demographic data (e.g., age, sex, body mass index (BMI), associated drug therapy, comorbidities (e.g. dyslipidaemia, arterial hypertension, cardiovascular diseases, chronic kidney disease, chronic respiratory diseases, diabetes mellitus, hypothyroidism, etc.) were all recorded. Also, recorded were the date of hospital admission, date of Nibima (investigational product) administration and dates of discharge or death. Additionally, we recorded the WHO score (8-point clinical progression scale) (**Table 1**) at hospital admission, at 24 hours, 7 days, 14 days post IP administration (where applicable), and at hospital discharge.

Table 1: WHO ordinal scale for clinical improvement**.**

	Score
Uninfected	
No clinical or virological evidence of infection	0
Ambulatory	
No limitations of activites	1
Limitation of activities	2
Hospitalised (mild disease)	
Hospitalised (no oxygen therapy)	3
Oxygen by mask or nasal prongs	4
Hospitalised (severe disease)	
Non-invasive ventilation or high-glow oxygen	5
Intubation and mechanical ventilation	6
Ventilation plus additional organ support: pressors, renal replacement therapy, extracorporeal membrane oxygenation	7
Dead	
Death	8

Randomisation

The study employed an open-label, randomised controlled phase II pilot clinical trial in recruiting patients. Before giving their consent, our clinical research personnel carefully verified the patients' eligibility. The participants were allocated to two treatment groups (the control group or the investigational product (IP) group) using a simple

randomisation method based on their pre-assigned unique personal identification number (PIN). Random numbers were generated for each PIN, and the allocation was performed using a computerised random number generator. A randomisation list was provided to the research personnel, showing the corresponding treatment group for each PIN. Therefore, following informed consent, each participant was assigned a PIN and the corresponding treatment group was identified from the randomisation list. Aqua (drinking water) and active treatment (Nibima) were all included in the randomisation sequence. However, both groups were put on the COVID-19 standard-of-care treatment approved by the Ghana Health Service. In compliance with regulatory standards, the IP was supplied in ready-to-use aqueous solutions in prelabelled syringes.

Procedures

Each time before dosing, the clinical team examined the patients in the domains of clinical frailty [mobility, energy, physical activity and function; vital signs (temperature, respiratory rate, heart rate, systolic blood pressure) and physical examination with clinical assessments guided by the WHO Ordinal Scale for Clinical Improvement (OSCI; **Table 1**). This was followed by blood samples, nasopharyngeal swabs, and sputum, which were collected and transported to the laboratory for analysis, see laboratory analyses section.

Nibima or aqua was administered at a dose of 30 ml, 8 hourly (thrice a day) after meals for up to 14 days. While patients were in the hospital during the treatment period, vital signs and levels of consciousness or evidence of confusion or agitation were recorded daily. The use of concomitant medications was also recorded throughout the study. Patients were regularly assessed for signs or symptoms that might be considered adverse events related to the IP use. When patients were discharged from the hospital during the study, the assessments were done by telephone.

Laboratory Analyses
Haematology and Blood Chemistry

The haematological indices (haemoglobin, haematocrit, red blood cell count, white blood cell count and platelet count) were assessed using the Sysmex XP-300 Automated Haematology Analyser (Sysmex Corporation, Kobe, Japan). In contrast, the liver function indices (aspartate transaminase, alanine transaminase, alkaline transaminase, gamma-glutamyl transferase, total protein, albumin, globulin and total bilirubin) and kidney function indices (urea and creatinine) were assessed using the BT 3500 Biochemistry Analyser (Diamond Diagnostics Inc., Holliston, USA).

Immunological assays

The serum levels of interleukin 6 (IL-6) were estimated using the Human IL-6 High Sensitivity ELISA (Cat #: BMS213HS). Also, the serum levels of C-reactive proteins (CRP) were evaluated using the Human C-reactive protein ELISA Kit (Cat # KHA0031). In addition, the serum levels of D-Dimer were assessed using the Human D-DIMER ELISA Kit (Cat # EHDDIMER). All these kits were purchased from Invitrogen and were used by following the manufacturer's instructions.

Assessment of viral load

For all patients enrolled in this study, oropharyngeal swabs were collected using standard oropharyngeal swabs. Samples were then transported in a viral transport medium via cold chain to the Kumasi Centre for Collaborative Research in Tropical Medicine for testing. RNA was extracted, and PCR was performed using DaAnGene PCR kits (Guangdong, China). The PCR cycling conditions were 50^{o}C for 15 mins for reverse transcription, 95^{o}C for 1 min for denaturation and 45 cycles of 95^{o}C for 15 seconds and 55^{o}C for 45 seconds. All samples with cycling thresholds above 40 were considered negative. Viral load was extrapolated from a standard curve generated from plotting known viral concentrations against cycling thresholds for a single target. All PCRs were validated using positive and negative controls. A SARS-CoV-2 positive sample was used as a positive control, whereas nuclease-free water was used as a negative control.

Outcome measurements

The primary endpoints were improved clinical recovery coupled with reduced hospital stay and reduced viral load, whereas the secondary endpoints were improved haematological, biochemical and immunological indices. Throughout the study period, patients frequently underwent non-suggestive questioning about their well-being and any adverse events they experienced were noted.

Sample size and study power estimate

A randomised control open-label trial proposes to evaluate the efficacy of Nibima in reducing both COVID-19 viral load and hospitalisation among the study participants. The sample size calculation was based on the primary hypothesis of detecting a reduction of 50% in viral loads of COVID-19 and reduced hospital stays between the treatment and control groups. In a randomised controlled trial (RCT) with a continuous outcome (i.e., viral loads), comparing mean outcomes between the two groups, the number of subjects per group for a two-sided significance level α (5%) and power $1 - \beta$ (80%) is given by:

$n = 2[Z1\text{-}\alpha/2 + Z1\text{-}\beta]\ 2/\Delta 2;$

where $\Delta 2 = (\mu T - \mu C)/\ \sigma$

(source: Campbell and Walters, 2014)

Where:

n = sample size required in each group,

Δ^2 is the standardised effect size. From Zhou et al. (2020), we assume a standardised effect size of 0.83

$Z_{1-\alpha/2}$: is the appropriate value from the standard normal distribution for the 100 (1-α/2) percentile, for 5%, this is 1.96

$Z_{1-\beta}$: is the appropriate value from the standard normal distribution for the 100 (1- β) percentile, which is the power for 80% is 0.84

Based on the above formula, with a power of 80%, a 95% confidence level, an effect size of 0.83, and considering a dropout rate of 20%, the sample size was estimated to be approximately 24 cases in each group. Thus, 48 patients in total.

Statistical Analysis

The data were collected using paper questionnaires, which were then entered into the REDCap electronic platform and exported to Excel spread sheet for analysis. The primary outcome analysis and other baseline data of patients enrolled were tabulated. The Kolmogorov-Smirnov test was used to evaluate the normality of the data distribution. Continuous variables were summarised as means with standard deviations (mean ± SD) at each follow-up period, whereas categorical variables were summarised by frequency and percentages for each follow-up period. Repeated measures analysis of variance (ANOVA) was used to assess the changes in HIV viral loads and other parameters over time, accounting for the within-subject correlation of repeated measurements and determining significant differences between the follow-up periods by potentially adjusting for covariates if necessary. Categorical variables were analysed using the chi-square or Fisher's exact test. Missing values were excluded from the statistical analysis. Using a two-sided test with a significance level of 5%, *p*-values less than 0.05 were considered statistically significant. All the statistical analyses were performed using STATA version 15.

Ethical Clearance

The trial protocol was reviewed and approved by the following entities: (1) the Committee for Human Research, Publication and Ethics (CHRPE) of the School of Medicine and Dentistry of the Kwame Nkrumah University of Science and Technology (CHRPE/AP/330/20); (2) the Clinical Trial Unit of the Ghana Health Service Ethics Review Committee (GHS/RDD/ERC/Admin/App/20/510); and (3) the Clinical Trial Department of the Food and Drugs Authority of Ghana (FDA/CT/211). Also, the trial was registered with the Pan African Clinical Trial Registry (PACTR 202010690466874). All patients or carers for those with severe illness consented to the study either via verbal or written consent. Safety data were reviewed and monitored by an independent data safety monitoring committee.

Results

Demographic characteristics, symptoms and comorbidities among study participants

A total of 42 patients consented to take part in this study. However, 40 patients who satisfied the inclusion criteria were enrolled in the study. Of the 40 patients, 4 voluntarily withdrew from the study, and 18 had incomplete data. Hence, out of the 40, data from 18 patients were included in the statistical analysis at baseline. Most of the 18 patients were males (n=11; 61.1%), and the majority were aged 46-59 years and above (83.3%) (**Table 2**). Concerning the symptoms of COVID-19, most patients presented with cough, fatigue, sputum production, headache and myalgia (**Table 3**). Moreover, a few patients had underlying conditions such as hypertension, diabetes, asthma, chronic liver disease and human immunodeficiency virus infection (**Table 4**).

Table 2: Demographic characteristics of study participants.

Demographic Characteristics	Intervention n (%)	Control n (%)	Total N
Sex			
Male	5 (45.45)	6 (54.55)	11
Female	4 (57.14)	3 (42.86)	7
Age			
18 – 45	0 (0.00)	3 (100.00)	3
46 – 59	5 (71.43)	2 (28.57)	7
>59	4 (50.00)	4 (50.00)	8

Table 3: COVID-19 symptoms reported by participants

Symptoms	Intervention n (%)	Control n (%)	Total N
Fever			
Yes	5(55.56)	4(44.44)	9
No	4(44.44)	5(55.56)	9
Cough			
Yes	9(56.25)	7(43.75)	16
No	0(0.00)	2(100.00)	2
Sore throat			
Yes	4(44.44)	5(55.56)	9
No	5(55.56)	4(44.44)	9
Painful swallowing			
Yes	0(0.00)	5(100.00)	5
No	9(69.23)	4(30.77)	13
Runny Nose			
Yes	4(44.44)	5(55.56)	9
No	5(55.56)	4(44.44)	9
Chest Pain			
Yes	3(33.33)	6(66.67)	9
No	6(66.67)	3(33.33)	9
Diarrhoea			
Yes	2(25.00)	6(75.00)	8
No	7(70.00)	3(30.00)	10
Fatigue			
Yes	9(50.00)	9(50.00)	18
No	0(0.00)	0(0.00)	0
Sputum production			
Yes	5(41.67)	7(58.33)	12
No	4(66.67)	2(33.33)	6
Headache			
Yes	7(43.75)	9(56.25)	16
No	2(100.00)	0(0.00)	2
Myalgia			
Yes	6(46.15)	7(53.85)	13
No	3(60.00)	2(30.00)	5

Table 4: Comorbidities recorded among participants.

Comorbidities	Intervention n(%)	Control n(%)	Total
Chronic cardiac disease			
Yes	0(0.00)	0(0.00)	0
No	9(50)	9(50)	18
Hypertension			
Yes	2(22.22)	7(77.78)	9
No	7(77.78)	2(22.22)	9
Diabetes			
Yes	3(75.00)	1(25.00)	4
No	6(42.86)	8(57.14)	14
Asthma			
Yes	0(0.00)	2(100.00)	2
No	9(56.25)	7(43.75)	16
Chronic kidney disease			
Yes	0(0.00)	0 (0.00)	0
No	9(50.00)	9 (50.00)	18
Chronic liver disease			
Yes	0(0.00)	1(100.00)	1
No	9(52.94)	8 (47.06)	17
HIV			
Yes	0(0.00)	1(100.00)	1
No	9(60.00)	6(40.00)	15

Number of deaths and days of clinical recovery among study participants

The study participants in the intervention arm recovered two days earlier compared to those in the control aqua arm, i.e., 5 out of 9 within 4-11 days and 3 out of 9 within 6-13 days, respectively. Also, 1 patient died in the intervention arm on day 8, whereas 4 died in the control arm on days 2, 4, 6 and 7. Five (5) recovered patients (intervention arm: 3 and control arm: 2) who were discharged were lost to follow-up (**Table 5)**.

Table 5: Loss-to-follow-up, death and recovery analysis

Visit/Death/Recovery	Intervention n (%)	Control n (%)	Total N
Baseline	9(50.00)	9(50.00)	18
Visit 1	6(50.00)	6(50.00)	12
Visit 2	5(62.50)	3(37.50)	8
Death	1(20.00)	4(80)	5
	Mean* (95% CI)	**Mean* (95% CI)**	
Recovery date	7.56 (4, 11)	9.89 (6.4, 13.4)	

**The unit is the number of days taking to recover; CI: confidence interval*

Haematological, kidney and liver function indices among study participants

Except for the haematocrit, there was no statistical difference in all the patients' haematological indices between the treatment groups and the baseline and first follow-up visits (**Table 6**). Similarly, except for the alanine transaminase activity, there was no statistical difference in all the liver function indices compared between the treatment groups and the baseline and first follow-up visits among the patients (**Table 7**). Also, there was no statistical difference in the patients' kidney function indices between the treatment groups (**Table 8**).

Table 6: Haematological indices among study participants using repeated measures ANOVA.

Haematological Index	Visit	Intervention Mean (SE)	Control Mean (SE)	F(*p*-value)
Haemoglobin (g/dL)	0	11.32(1.10)	12.78(1.08)	1.11(0.32)
	1	10.82(1.13)	12.58(1.26)	
Haematocrit (%)	0	40.71(3.15)	39.73(3.10)	21.15(<0.01)*
	1	33.95(3.21)	46.83(3.50)	
Red blood cell count (10^6/uL)	0	3.91(0.38)	4.55(0.37)	1.67(0.22)
	1	3.75(0.39)	4.49(0.45)	
White blood cell count (10^3/uL)	0	7.20(1.42)	8.92(1.35)	5.11(0.05)
	1	5.62(1.53)	12.57(1.96)	
Platelet count (10^3/uL)	0	207.86(36.40)	233.00(36.18)	2.71(0.13)
	1	206.77(36.73)	350.53(38.13)	

**p < 0.05; 0: Baseline; 1: Visit 1; SE: Standard of error*

Table 7: Liver function indices among the study participants using repeated measures ANOVA.

Liver Function Index	Visit	Intervention Mean (SE)	Control Mean (SE)	F(*p*-value)
Aspartate transaminase (U/L)	0	63.38(10.29)	33.97(9.48)	3.22(0.10)
	1	35.78(11.40)	17.96(15.74)	
Alanine transaminase (U/L)	0	140.31(50.67)	81.75(47.04)	0.82(0.39)
	1	27.96(55.68)	2.37(75.41)	
Alkaline transaminase(U/L)	0	210.78(38.22)	95.08(36.19)	4.32(0.06)*
	1	176.78(176.78)	78.38(52.48)	
Gamma-glutamyl transferase (U/L)	0	80.03(17.65)	63.63(16.55)	1.02(0.34)
	1	69.52(19.19)	38.49(25.26)	
Total protein (g/L)	0	62.13(11.94)	62.48(10.90)	0.03(0.87)
	1	74.08(13.35)	69.01(18.88)	
Albumin(g/L)	0	37.87(4.62)	43.42(4.25)	0.95(0.35)
	1	43.94(5.11)	47.72(7.07)	
Globulin (g/L)	0	24.18(8.41)	22.44(7.69)	0.15(0.70)
	1	34.15(9.39)	28.43(13.24)	
Total Bilirubin (µmol/L)	0	3.41(1.77)	5.66(1.66)	0.02(0.90)
	1	5.67(1.92)	4.04(2.52)	

0: Baseline; 1: Visit 1; SE: Standard of error

Table 8: Kidney function indices among the study participants using repeated measures ANOVA.

Kidney Function Index	Visit	Intervention Mean (SE)	Control Mean (SE)	F(*p*-value)
Urea (mmol/L)	0	2784.78(1420.90)	3318.63(1384.62)	0.32(0.59)
	1	1993.42(1473.65)	3683.75(1690.64)	
Creatinine (µmol/L)	0	1365.20(6508.94)	14524(6508.93)	2.05(0.18)
	1	1341.33(6508.94)	14511(6508.96)	
Urea-creatinine ratio	0	12.03(3.55)	11.17(3.27)	0.16(0.70)
	1	12.62(3.93)	17.06(5.43)	

0: Baseline; 1: Visit 1; SE: Standard of error

Inflammatory and coagulation markers among the study participants

There was no statistical difference in the inflammatory and coagulation markers between the patients' baseline and first follow-up visits and the treatment groups (**Table 9**). Also, there was no statistical difference in the viral load between the patients' baseline and first follow-up visits and the treatment groups (**Table 10**).

Table 9: Inflammatory and coagulation markers among the study participants using repeated measures ANOVA.

Inflammatory and Coagulation Marker	Visit	Intervention Mean (SE)	Control Mean (SE)	F(*p*-value)
C-reactive protein (mg/mL)	0	37.43(13.05)	59.45(12.77)	1.16(0.31)
	1	44.27(13.45)	61.34(15.12)	
Interleukin 6 (pg/mL)	0	905.80(709.82)	29.48(648.63)	1.56(0.24)
	1	1189.03(792.81)	41.76(1118.44)	
D-dimer (mg/mL)	0	31.11(14.69)	18.68(14.55)	0.88(0.37)
	1	20.29(14.89)	16.91(15.76)	

0: Baseline; 1: Visit 1; SE: Standard of error

Table 10: SARS-CoV-2 viral load among the study participants using repeated measures ANOVA.

Viral Load	Visit	Intervention Mean (SE)	Control Mean (SE)	F(*p*-value)
RNA copies/µL	0	7417.46(5682.08)	10663(6642.03)	0.01(0.94)
	1	2840.03(5682.08)	1607.80(7959.16)	

0: Baseline; 1: Visit 1; SE: Standard of error

Discussion

This study was a randomised, controlled open-labelled phase-II trial to evaluate the efficacy and safety of Nibima (aqueous extract of *Cryptolepis sanguinolenta*) in patients admitted to a hospital with moderate to severe COVID-19. The results of this pilot trial have shown that Nibima, when administered at 30 ml thrice daily after meals for about 14 days, was associated with more rapid clinical recovery and less hospital stay compared with COVID-19 patients in the control arm who did not take the Nibima (OSCI ≤1). Furthermore, 1 death (OSCI =8) was recorded in the patients administered with Nibima on day 8, whereas 4 deaths (OSCI =8) were recorded in the control arm (were not administered Nibima) on days 2, 4, 6 and 7. These results imply a reduction in the risk of progression to severe disease or death in the patients who were administered the Nibima. The current study also suggested that Nibima was safe as no patient reacted adversely to it. The safety of Nibima was further consolidated by the results of the secondary outcome analysis of the patients' haematological, liver and kidney function indices that were within the normal reference ranges (Tables 6, 7 and 8).

Results from our preclinical studies indicated that cryptolepine (an active ingredient of Nibima) demonstrated broad-spectrum antiviral and immunomodulatory activities through the augmentation of the type I interferon response pathway (Domfeh *et al.,* 2021a; Domfeh *et al.,* 2021b; Domfeh *et al.,* 2022). Interferons are natural antiviral proteins released by host cells in response to the presence of viruses. Indeed, we previously showed that Nibima inhibited hepatitis B virus replication (Domfeh *et al.,* 2021b). Some ongoing randomised controlled trials are seeking to explore the effects of injected type I interferons in COVID-19 (Hung *et al.,* 2020). In 2023, Reis *et al.,* (Reis *et al.,* 2023) reported that early treatment with pegylated interferon lambda reduced COVID-19 hospitalisations consistent with the results of the current study. Also, in consistent with our results, a randomised, double-blind, placebo-controlled phase II trial reported that patients who inhaled nebulised interferon beta-1 had greater odds of improvement and recovery from SARS-CoV-2 infection than those who received a placebo (Monk *et al.,* 2021). In this previous study, three deaths were recorded in the placebo group and none in the treatment group (Monk *et al.,* 2021). This outcome is consistent with our results, where four deaths were recorded in the control arm and one in the intervention arm. Furthermore, herbal medicines have been shown to improve the symptoms of COVID-19 patients in randomised control trials (Ang *et al.,* 2020; Li *et al.,* 2020; Lee *et al.,* 2021; Kumar *et al.,* 2022), indicating that herbal medicines can be exploited for COVID-19 management. Taken together, there is compelling evidence that interferon-based therapy is effective and safe in managing COVID-19, and Nibima is a very promising anti-COVID-19 drug.

Although the current study did not report a reduction in COVID-19 viral load, it is conceivable that Nibima's ability to induce interferon production coupled with its immune-boosting properties could drive early recovery by limiting the viral replication to the upper respiratory tract (Mensah-Kane *et al.,* 2020). Another reason could be that Nibima directly inhibited SARS-COV-2 replication in a similar fashion as we previously reported (Borquaye *et al.,*

2020; Domfeh *et al.*, 2021a; Domfeh *et al.*, 2021b; Domfeh *et al.*, 2022). Interestingly, in low- and middle-income countries (LMICs), over 80% of people rely on herbal medicines for their primary health care needs, supporting the need for medicinal plants to manage COVID-19 patients in LMICs.

Our study had some limitations. The sample size was limited, making the generalisability of the findings to wider populations and healthcare systems challenging. In our clinical trial, not all the factors were randomised; hence, the intervention and control arms were not well matched for age, sex and comorbidities. Phase III trials will address these issues associated with randomising larger and more heterogeneous groups. Due to the urgency for homegrown interventions at the peak of the COVID-19 pandemic and to deliver the trial results, patients in our study were followed for fourteen days instead of longer. Also, some patients were lost to follow-up or refused follow-up calls due to perceived stigmatisation. These losses to follow-ups restricted our statistical analysis to baseline and the first follow-up data, which could account for the non-significant differences in the inflammatory markers and viral load in the patients. The results suggest Nibima as a promising drug that could effectively manage COVID-19 patients and further suggest a stronger rationale for larger studies of hospitalised patients with COVID-19 and other respiratory infections. However, further larger studies could be done for more than fourteen days to unravel the effects of Nibima on the inflammatory markers and viral load among hospitalised COVID-19 patients. Importantly, the current study strategy could be adopted for respiratory infections of viral origins, including influenza viruses.

Conclusion

Nibima, the anti-malarial herbal product, was well tolerated in hospitalised patients with moderate to severe COVID-19, with increased clinical recovery among the patients. The evidence from this study provides a strong rationale for larger studies in hospitalised patients with COVID-19 and other related respiratory infections. Although COVID-19 has been declared no more a pandemic, it is still a public health challenge with complications that could be life-threatening. Formulations of *C. sanguinolenta* products could, therefore, be further investigated for their broad-spectrum antiviral and immunomodulatory activities on viral infections in preparedness for re-emergence of future pandemics.

Data availability

All the data obtained and analysed are included in this manuscript.

Conflicts of interest

The authors declare that there is no conflict of interests associated with this study.

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