JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH

RESEARCH ARTICLE

Efficacy of Integrative Siddha Medicine, Kabasura Kudineer with Vitamin C, Zinc Supplementation for the Management of Asymptomatic COVID 19 Cases in Tertiary Hospital: A Randomized Controlled Trial

ANBARASI CHANDRASEKARAN[1*], SANKARARAJ PALANIBOMMU[2], VIJAYABASKAR PANDIAN[3], JEYAPRAKASH PANDIYARAJ[4], AMUDHAN MURUGESAN[5], REVATHI RAMAKRISNAN[6], LALLITHA SIVATHANU[7], PALANIKUMAR BALUCHAMY[8], VIJAYALAKSHMI MASILAMANI[9], JAYALAKSHMI MARAIAKUTTIKAN[10], PARTHIBAN P[11], KANAKAVALLI K[12], ILANGOVAN MUTHUKARUPPIAH[13]

[1]Central Council for Research in Siddha, Siddha Central Research Institute, Arumbakkam, Chennai, Tamil Nadu – 600106, India, Email: dranbu1208@gmail.com

[2]Govt. Theni Medical College & Hospital, Theni. Tamil Nadu -625512, India, Email: drsankararaj@gmail.com

[3]Multi-Disciplinary Research Unit (MDRU), Govt. Theni Medical College & Hospital, Theni. Tamil Nadu -625512, India, Email: baski_bos@yahoo.co.in

[4]Multi-Disciplinary Research Unit (MDRU), Govt. Theni Medical College & Hospital, Theni. Tamil Nadu -625512, India, Email: jpdhina@gmail.com

[5]Dept. of Microbiology, Govt. Theni Medical College & Hospital, Theni. Tamil Nadu -625512, India, Email: dramudhanmicro@gmail.com

[6]Multi-Disciplinary Research Unit (MDRU), Govt. Theni Medical College & Hospital, Theni. Tamil Nadu -625512, India, Email: revathiram67@gmail.com

[7]Dept. of Microbiology, Govt. Theni Medical College & Hospital, Theni. Tamil Nadu -625512, India, Email: gtmcmicrobiology@gmail.com

[8]Dept. of Medicine, Govt. Theni Medical College & Hospital, Theni. Tamil Nadu -625512, India, Email: drPK9396@gmail.com

[9]Dept. of Biochemistry, Govt. Theni Medical College & Hospital, Theni. Tamil Nadu -625512, India, Email: viji1310.masi@gmail.com

[10]Dept. of Immunology, School of Biological Sciences, Madurai Kamaraj University, Madurai.Tamil Nadu- 625021, India, Email: jayalakshmimk11@yahoo.com

[11]Directorate of Indian Medicine & Homeopathy, Arumbakkam, Chennai, TamilNadu-600106, India, Email: parniru@gmail.com

[12]Central Council for Research in Siddha, Arumbakkam, Chennai, TamilNadu-600106, India, Email: drkkanakavalli@gmail.com

[13]Govt. Theni Medical College & Hospital, Theni. Tamil Nadu -625512, India, Email: drilangovanms@gmail.com

**Corresponding Author*

ABSTRACT

Introduction: Non-tested asymptomatic COVID-19 cases poses threat of transmitting the disease silently. The Siddha polyherbal formulation, Kabasurakudineer, (KSK) was found to be effective in preventing viral replication of SARS-CoV-2 by in-silico studies. A pilot study was conducted to test the antiviral activity of KSK in asymptomatic individuals tested positive for COVID-19.

Methods: A single centre, open labelled, randomized controlled study was carried out during June-August 2020, in Tertiary Medical College Hospital, after approval from the institutional ethics committee and registered in CTRI. RTPCR confirmed COVID-19 asymptomatic cases, aged 18-65 years, consented to participate were included and those with co-morbidities like diabetes, hypertension, severe respiratory disease, malignancies, pregnant and lactating mothers were excluded. 60 participants were randomly assigned to study and control group. Study group received KSK (60 ml) along with vitamin C in the morning and zinc in the night, while the control group (CZ) received vitamin C and zinc for 10 days. The primary outcome was the reduction in the SARS-CoV-2 load (ct value), prevention of progression to symptomatic state.

Results: In the study group, there was faster reduction in the viral load in terms of ct value as all the 30 participants turned negative for SARS-Co-V2, while 4 remain positive in the control group on the 10th day. The inflammatory markers and serum cytokine findings were inconclusive. No one progressed to the symptomatic state and no adverse event was reported in either groups.

Conclusion: This study demonstrated the potential of Kabasurakudineer in reducing the viral load. Further clinical studies are warranted with larger sample size.

KEYWORDS:
Kabasura Kudineer, Siddha medicine, Integrative Medicine, Vitamin C, Asymptomatic COVID 19

ARTICLE HISTORY:
Received Oct 10, 2021
Accepted Nov 15,2021
Published Dec 20, 2021

DOI:
10.5455/jcmr.2021.12.04.38

VOLUME: 12
ISSUE: 4
ISSN: 2146-8397

INTRODUCTION

We have always been afflicted by infectious diseases, usually viral, leading to outbreaks, epidemics and rarely pandemics, during the past centuries. We have been now living with COVID 19, which emerged in December 2019 in China and was declared a pandemic in March 2020 by WHO. The devastating SARS-Co-V2 virus has infected millions of people across 200 countries with 79.2 million confirmed cases, that led to 1.7 million deaths till 27th December 2020, since the pandemic (1) struck. This created a global crisis worse than all previous epidemics and pandemics which cause gigantic morbidity and transience, regardless of extensive medical facilities. Majority of the patients are not aware of their disease and do not isolate or seek treatment, and unknowingly transmit the virus. Thus the undetermined asymptomatic COVID 19 positive cases pose a serious threat and could be a source of contagion (2). The main challenge in controlling the pandemic infection is the absence of approved drugs for the management of the disease.

Based on WHO Guidance on Monitored Emergency Use of Unregistered Interventions (MEURI) (3) various traditional, complementary and alternative medicines have been tried as a solution through clinical studies to find out a safe potential remedy (4) globally.

Ministry of AYUSH, Government of India came out with the advisory on 6th March 2020, for proper usage of AYUSH (Ayurveda, Yoga, Unani, Siddha, Homeopathy) medicines in managing the outbreak as a preventive care, symptomatic management and/or add on intervention to the conventional care. (5) In continuation of this, the Tamil Nadu state government released an order for implementing a special programme (AAROKYAM) with AYUSH interventions for COVID 19 on 23rd April 2020 (6).

One of the traditional formulations from Siddha system of medicine, Kabasura kudineer (KSK) consisting of 15 herbs, was included in the management of COVID-19 infection. This formulation, in an in-silico computational studies, showed that 9 phytoconstituents had high binding affinity against SARS-COV-2 Spike Protein (7). The preclinical studies, in-vitro/in-vivo studies carried out in KSK, showed that it possessed anti-pyretic, anti-inflammatory and anti-bacterial effects (8).

Govt. of Tamil Nadu created Isolation wards, COVID care centers to manage the positive asymptomatic patients and issued a guideline indicating administration of Vitamin C and Zinc (CZ) to all admitted cases. Later KSK was also included in the list of medicines administered in isolation ward and COVID care centers. Therefore the integration of KSK and CZ initiated in the isolation wards and COVID care centers. There is no prior studies in the evaluation of integrative medicine in the management of COVID 19 asymptomatic cases. (9).

Therefore, we propose to compare the effect of KSK and CZ in terms of reduction in viral load of SARS CoV- 2 infection, prevention of progression of asymptomatic to symptomatic state and effect on immune system in terms of selected immunological markers.

METHODS

Study design

The study was a randomized open labelled controlled trial in a parallel group with 1:1 allocation ratio.

Study Participants

The participants were asymptomatic positive cases. Through contact tracing, the health authorities identified high risk contacts in the Theni district and screened them for SARS-Co-V2, using RTPCR test performed on nasopharyngeal and oropharyngeal swabs.

Study setting

The study was done at Government Theni Medical College Hospital.

Inclusion criteria

RTPCR confirmed COVID 19 asymptomatic cases, of both male and female gender, aged 18-65 years who consented to participate were included

Exclusion criteria

Patients with co-morbidities like diabetes mellitus, hypertension and bronchial asthma, or with severe respiratory diseases and malignant diseases were not included. Pregnant women and lactating mothers were also excluded.

Intervention

The study groups (KCZ) were given Kabasura kudineer (60 ml) orally after food along with vitamin C(100mg) and zinc (20mg), in the morning and evening, respectively, while the control group (CZ) received vitamin C (100mg) and Zinc (20 mg) supplementation in the morning and evening, respectively, for 10 days.

The study drug Kabasura kudineer, is a polyherbal formulation comprising 15 herbs, namely Zingiber officinale Rosc., Piper longum L., Syzygium aromaticum (L.) Merr. & Perry, Anacyclus pyrethrum (L.) Lag., Tragia involucrata L., Hygrophila auriculata (Schum.) Heine Lam., Terminalia chebula Retz, Justicia adhatoda L., Plectranthus amboinicus (Lour.) Spreng, Costus speciosus (J. Koenig) Sm., Tinospora sinensis (Lour.) Merr., Clerodendrum serratum (L.) Moon, Andrographis paniculata (Burm.f.) ex Nees, Cyperus rotundus L. and Cissampelos pareira L. The drug (coarse powder) was procured from Tamil Nadu Medicinal Plants Corporation Limited (TAMPCOL), a GMP certified company, run by the Government of Tamil Nadu, India. The decoction was prepared by boiling 5g of KSK Chooranam in 240 ml water and reduced to one-fourth (60ml) and filtered. This freshly prepared 60ml decoction was given to each participant after food, orally, both in the morning and in the evening.

Outcome Measures

The primary endpoint was the reduction of SARS-CoV-2 viral load in terms of cycle threshold (ct) value in RT-PCR, prevention of progression of asymptomatic to symptomatic state and immunological assessment from the baseline. The secondary endpoint was documenting any adverse effects or event, if any, during the study period.

At the time of recruitment, details on the socio-demographic information were collected. Recording the vital signs, oxygen saturation (SpO2) and clinical assessment were carried out during the entire study period. Every day, during the study period, the participants were assessed for drug compliance, any complaints, or adverse events. Clinical examination was performed in both allopathy and siddha system and relevant details collected according to the systems.

The biochemical assessment of renal function, liver function, hemogram, electrolytes, inflammatory markers, nasopharyngeal (NP) and oropharyngeal (OP) swab for RT-PCR test were done at the baseline and 10th day. The NP/OP swabs were used to detect viral RNA and quantification of ORF-gene, E-gene and S-gene was done by cycle threshold (ct) value in RT-PCR. The BD™ CBA Human Th1/Th2/Th17 Cytokine Kit was used to measure Interleukin-2 (IL-2), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor Necrosis Factor (TNF), Interferon-gamma and Interleukin-17 protein levels in a single sample using CytoFLEX instrument.

Sample size

Being an exploratory study, 30 participants in each arm, totally 60 was the sample size.

Randomisation

All the eligible 60 participants were randomly assigned (in 1:1 ratio), 30 in each arm to ensure balanced distribution between study group and control group. Randomization was done using www.randomizer.org and implemented by independent statistician who was not involved in data analysis. Equal numbers of cards with each arm assignment number randomly generated was placed in sequentially numbered envelops was opened by study participant during enrolment. Participants in the study group were given Kabasura Kudineer(KSK) along with Vitamin C and Zinc, while the control group received vitamin C and Zinc.

Statistical analysis

The categorical variables were presented in number (percentage) while the continuous variables were presented in mean (SD) or median (range). Independent sample t-test

was carried out for testing the mean difference of the study group and also adjusted mean difference using analysis of covariance (regression) using SPSS (version 20) software.

Ethics approval

"Ethics approval was obtained from Institutional Human Ethics" Committee of Government Theni Medical College Hospital, Theni, Tamil Nadu, India. The Trial was registered in Clinical Trial Registry of India (CTRI/2020/06/025874). Voluntary, written, informed consent was obtained from all eligible participants.

RESULT

The study was conducted during June-August 2020. 78 SARS-Co-V2 positive asymptomatic cases were screened, of whom 60 eligible participants were randomised to study group (KCZ) and control group (CZ), with 30 in each group (Figure 1). Both the study and control groups were almost similar in the baseline characteristics (Table 1 & 2).

All the participants were asymptomatic at the baseline and did not develop any symptom during the study period. There was no dropout of participants during the study period.

The RT-PCR for SARS-CoV-2 from nasopharyngeal and oropharyngeal swab turned negative for all 30 cases in the study group at the end of 10th day. In control group, 26 participants turned negative on 10th day while the remaining 4 on the 14th day.

There was significant decrease in the viral load in terms of ct value in the study group after treatment with adjusted baseline in all the three gene type - ORF gene, E gene and S gene with $p<0.05$. We conducted this study to explore the efficacy of integrative medicine (Kabasura kudineer with Vitamin C and Zinc) against that of Vitamin C and Zinc supplementation alone in the asymptomatic COVID cases. We found that Kabasura kudineer intervention accelerated the reduction in viral load when compared to the control group after adjusting the baseline value using linear regression. (Table 3).

The renal and liver function parameters values were well within the normal limit, though show statistical significant ($p<0.05$) in few parameters like alkaline phosphatase, total protein, albumin, total bilirubin, direct bilirubin and indirect bilirubin which is not clinically significant. We did not observe any statistical significant reduction in the inflammatory markers like CRP, D-dimer and Ferritin level at the end of the 10th day of treatment (Table 4).

The serum cytokine level IL2, IL4, IL6, IL10, IL17, TNF-alpha, IFN gamma studies were done at the baseline and end line of the treatment. The findings were inconclusive (Table 5).

We did not observe any changes in the Siddha-based clinical examination parameters. There were no adverse events reported in both the groups.

DISCUSSION

Our study found that the integrative treatment of KSK along with vitamin C and Zinc supplementation reduced the viral load of SARS-CoV-2 significantly in asymptomatic COVID 19 positive cases within 10 days. All the positive cases in the study group turned negative for SARS-Co-V2 in RT-PCR swab test on the 10th day, while 4 remained positive in the control group on the 10th day. It was observed that there is no disease progression from asymptomatic state in both the groups.

Distribution of asymptomatic patients in the community and the duration of virus elimination plays a vital role in the infectivity of these patients. Finding out asymptomatic patients in the community is difficult as they do not have any obvious clinical symptoms. Rigorous contact tracing and follow ups were used to identify these patients (10). Asymptomatic patients contribute in the rapid and extensive spread of SARS-CoV-2 infections (11) .

Estimated risk of transmission was highest in the first 5 days of illness (12). The median communicable period, defined as the interval from the first day of positive nucleic acid tests to the first day of continuous negative tests was 9.5 days (13). Median of onset of symptoms in asymptomatic is 4 days (14). Increasing evidence on transmission of SARS CoV2 by asymptomatic patients burdens the control of pandemic. Govt of Tamil Nadu has taken strenuous effect to control the transmission of SARS-CoV-2 from asymptomatic cases by adopting integrative treatment of KSK along with Vitamin C and Zinc tablets. The effect of this integrative treatment was not estimated until this study was conducted. Fortunately, this study revealed that KSK along with Vitamin C and Zinc has reduced viral load in terms of ct value and accelerated the recovery of the cases. This result also support the earlier findings on KSK in controlling the transmission potential (15-16).

Moreover, KSK has been identified as immunomodulating drug as it possessed phytochemicals like flavonoids, alkaloids, glycosides, diterpenoids (17). In-vitro studies on Andrographis paniculate, one of the ingredients of KSK, showed immunomodulatory activity as evidenced by the reduction of IL12, TNF alpha, NO, PGE2, COX-2 and iNOS in microglia and macrophages (18). Regulation of IFN gamma, IL2 and TNF alpha and andrographolide can modulate the innate immune response and regulate the antibody production (19). The phytoconstituents, cordiofolioside A, cordiofolioside B, syringin present in Tinospora cordifolia possess immunomodulatory activity.(20). In-vitro study on Zingiber officinale's immunomodulatory effect and its role as a potent anti-viral agent (21) were observed. Other ingredients of KSK, Adathoda vasica, Costus speciosus, Clerodendrum serratum and Anacyclus pyrethrum showed there was an improvement in the humoral and cellular immunity (22–25); in-vivo study on wistar rats showed that Cyperus rotundus modulated both cell-mediated and antibody-mediated immune responses (26). Another herb, Syzygium aromaticum, in an animal study,

showed that its water extract inhibited macrophages, to produce both IL-1beta and IL-6 (27). Although many of the herbs in kabasura kudineer demonstrated immunomodulatory activity in preclinical studies, the findings on the cytokine levels were not apparent in this clinical study.

On the otherside, increased inflammatory reaction is considered as host immune response for any viral infection. Normal anti-viral immune response entails the activation of inflammatory pathways, but exaggerated or aberrant response can cause severe disease, if not controlled. Both pro-inflammatory cytokines (IL-6, TNF-a, Interferon-gamma and IL-17) and anti-inflammatory cytokines (Ll-10) are elevated in cytokine storm COVID patients. (28,29). Interestingly, In this study, there was reduction in the level of TNF-alpha from baseline with median value 46.5pg/ml to endpoint 13.0pg/ml in study group when compared to the control group. This showed the potential of KSK in reducing the risk of aberrant inflammatory response and might be influencing the severity of infection. However, insignificant reduction in the CRP level (0.96 mg/L) (p=0.09) in study group and small sample size are not sufficient to arrive a conclusion. Hence, it warrants for further investigation in the line of anti inflammatory action with larger sample size.

Serum ferritin level increases during viral infection and inflammation. It was found that it was the last laboratory value to return to normal compared to the other active proteins ,like c-reactive protein (30). Ferritin in COVID 19 patients is screened to evaluate the presence of hyper inflammation and to predict the worsening of the patient's health condition (31). It is a key mediator of immune dysregulation via direct immune suppressive and proinflammatory effects contributing to the cytokine storm, as it might be a crucial factor influencing the severity of COVID. In our study, though there was a mean reduction of ferritin level (5.93ng/ml) in the study group, but not statistically significant (p=0.64).

CONCLUSION

The study demonstrated that Kabasura kudineer (KSK) may aid in reducing the viral load of SARS-Co-V2 and accelerated recovery. However, further clinical studies, with a large sample size, are warranted for establishing Kabasura kudineer in the management of SARS-Co-V2 infection both in asymptomatic and symptomatic conditions

This study had few limitations. Kabasura kudineer was evaluated as an add on therapy along with standard of care and not as a standalone therapy. Being exploratory study the sample size was small and also difficulty in recruiting asymptomatic positive cases, lack of quantitative viral load detection using q-RTPCR, due to non-availability of the resources at the study centres. Hence, larger multi-centric study with sufficient power to detect assumed differences in reduction of viral load of SARS-CoV-2 and the impact on immunological markers, is warranted.

FUNDING

The study was primarily funded by Government Theni Medical College and Hospital, Tamil Nadu, India and partly by Central Council for Research in Siddha, Chennai, India.

ACKNOWLEDGEMENT

We would like to thank all the clinical, paramedical, and technical staff of Government Theni Medical College and Hospital and Multi-disciplinary Research Unit (MDRU) for their support, all the participants who agreed to participate in the study. We sincerely thank V. Sundara Bharathi, Associate Professor (English) and Academic Secretary, Velankanni Group of Institutions, Chennai for language review, Dr. V. Saravana kumar, Scientist B, NIE-ICMR for his valuable inputs in the statistical part of the manuscript and Vaidya P. Rammanohar, Research Director, Amirtha Vishwa Vidyapeetham, Kerala, for the critical reading of the manuscript.

Competing Interest

The authors have no competing interests.

REFERENCES

1. Weekly epidemiological update - 29 December 2020 [Internet]. [cited 2021 Jan 10]. Available from: https://www.who.int/publications/m/item/weekly-epidemiological-update---29-december-2020
2. Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, et al. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany [Internet]. New England Journal of Medicine. Massachusetts Medical Society; 2020 [cited 2021 Jan 10]. Available from: https://www.nejm.org/doi/10.1056/NEJMc2001468
3. notes-for-the-record-meuri-ebola.pdf [Internet]. [cited 2021 Jan 18]. Available from: https://www.who.int/ebola/drc-2018/notes-for-the-record-meuri-ebola.pdf
4. Luo E, Zhang D, Luo H, Liu B, Zhao K, Zhao Y, et al. Treatment efficacy analysis of traditional Chinese medicine for novel coronavirus pneumonia (COVID-19): an empirical study from Wuhan, Hubei Province, China. Chin Med. 2020 Dec;15(1):34.
5. 125.pdf [Internet]. [cited 2021 Jan 18]. Available from: https://www.ayush.gov.in/docs/125.pdf
6. AAROKYAM.pdf [Internet]. [cited 2021 Jan 8]. Available from: https://stopcorona.tn.gov.in/wp-content/uploads/2020/03/AAROKYAM.pdf
7. Gangarapu K, Karthik L, Devi M S S, Parameswaran S, Kanakavalli K, Kumar KM, et al. In Silico computational screening of Kabasura Kudineer - Official Siddha Formulation and JACOM against SARS-CoV-2 spike protein. J Ayurveda Integr Med. 2020 May 1;
8. Vincent S, Arokiyaraj S, Saravanan M, Dhanraj M. Molecular Docking Studies on the Anti-viral Effects of Compounds From Kabasura Kudineer on SARS-CoV-2 3CLpro. Front Mol Biosci [Internet]. 2020 Dec 23 [cited 2021 Jan 10];7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7785853/

9. Tamil Nadu (Archive) – StopCoronaTN [Internet]. [cited 2021 Jan 10]. Available from: https://stopcorona.tn.gov.in/government-of-tamil-nadu-archive/
10. Rodrigues TG, Fernandes A, Sousa JPB, Bastos JK, Sforcin JM. In vitro and in vivo effects of clove on pro-inflammatory cytokines production by macrophages. Nat Prod Res. 2009;23(4):319–26.
11. Spread of SARS-CoV-2 in the Icelandic Population | NEJM [Internet]. [cited 2021 Sep 24]. Available from: https://www.nejm.org/doi/full/10.1056/NEJMoa2006100
12. Long Q-X, Tang X-J, Shi Q-L, Li Q, Deng H-J, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020 Aug;26(8):1200–4.
13. He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med. 2020 May;26(5):672–5.
14. Yang R, Gui X, Xiong Y. Comparison of Clinical Characteristics of Patients with Asymptomatic vs Symptomatic Coronavirus Disease 2019 in Wuhan, China. JAMA Netw Open. 2020 May 27;3(5):e2010182.
15. Natarajan S. Kabasura Kudineer (KSK), a poly-herbal Siddha medicine, reduced SARS-CoV-2 viral load in asymptomatic COVID-19 individuals as compared to vitamin C and zinc supplementation: findings from a prospective, exploratory, open-labeled, comparative, randomized controlled trial, Tamil Nadu, India. 2021;11.
16. Srivastava A. Efficacy of two siddha polyherbal decoctions, Nilavembu Kudineer and Kaba Sura Kudineer, along with standard allopathy treatment in the management of mild to moderate symptomatic COVID-19 patients—a double-blind, placebo-controlled, clinical trial. 2021;11.
17. Jantan I, Ahmad W, Bukhari SNA. Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. Front Plant Sci [Internet]. 2015 [cited 2021 Jan 19];6. Available from: https://www.frontiersin.org/articles/10.3389/fpls.2015.00655/full
18. Jantan I, Ahmad W, Bukhari SNA. Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. Front Plant Sci [Internet]. 2015 [cited 2020 Jul 26];6. Available from: https://www.frontiersin.org/articles/10.3389/fpls.2015.00655/full
19. Shen Y-C, Chen C-F, Chiou W-F. Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect. Br J Pharmacol. 2002;135(2):399–406.
20. Maurya R, Wazir V, Kapil A, Kapil RS. Cordifoliosides A and B, two New Phenylpropene Disaccharides from Tinospora cordifolia possessing Immunostimulant Activity. Nat Prod Lett. 1996 Feb 1;8(1):7–10.
21. Immunopharmacological Activity of Zingiber officinale on Human Peripheral Blood Mononuclear Cells. Asian J. Med. Pharm. Res., 5 (2): 13-17. 2015;5.
22. Vinothapooshan G. Immunomodulatory activity of various extracts of Adhatoda vasica Linn. in experimental rats. Afr J Pharm Pharmacol. 2011 Mar 31;5(3):306–10.
23. IMMUNOSTIMULATORY POTENTIAL OF N-BUTANOLIC FRACTION OF HYDROALCOHOLIC EXTRACT OF COSTUS SPECIOSUS KOEN. RHIZOME | INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH [Internet]. [cited 2020 Jul 27]. Available from: https://ijpsr.com/bft-article/immunostimulatory-potential-of-n-butanolic-fraction-of-hydroalcoholic-extract-of-costus-speciosus-koen-rhizome/?view=fulltext
24. Kumar GMS. Evaluation Changes of Lungs in Asthmatic Rats Treated with Aqueous and Non Aqueous Extract of Clerodendrum Serratum and Theophylline. [Internet] [Master's Thesis]. Cherraan?s College of Pharmacy, Coimbatore; 2014. Available from: http://repository-tnmgrmu.ac.in/3728/
25. Sharma V, Thakur M, Chauhan NS, Dixit VK. Immunomodulatory activity of petroleum ether extract of Anacyclus pyrethrum. Pharm Biol. 2010 Nov;48(11):1247–54.
26. Seo W-G, Pae H-O, Oh G-S, Chai K-Y, Kwon T-O, Yun Y-G, et al. Inhibitory effects of methanol extract of Cyperus rotundus rhizomes on nitric oxide and superoxide productions by murine macrophage cell line, RAW 264.7 cells. J Ethnopharmacol. 2001 Jun 1;76(1):59–64.
27. Cortés-Rojas DF, de Souza CRF, Oliveira WP. Clove (Syzygium aromaticum): a precious spice. Asian Pac J Trop Biomed. 2014 Feb 1;4(2):90–6.
28. Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 Cytokine Storm; What We Know So Far. Front Immunol. 2020 Jun 16;11:1446.
29. Ghazavi A, Ganji A, Keshavarzian N, Rabiemajd S, Mosayebi G. Cytokine profile and disease severity in patients with COVID-19. Cytokine. 2021 Jan 1;137:155323.
30. Li Y, Shi J, Xia J, Duan J, Chen L, Yu X, et al. Asymptomatic and Symptomatic Patients With Non-severe Coronavirus Disease (COVID-19) Have Similar Clinical Features and Virological Courses: A Retrospective Single Center Study. Front Microbiol. 2020 Jun 26;11:1570.
31. Cheng L, Li H, Li L, Liu C, Yan S, Chen H, et al. Ferritin in the coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis. J Clin Lab Anal. 2020;34(10):e23618.

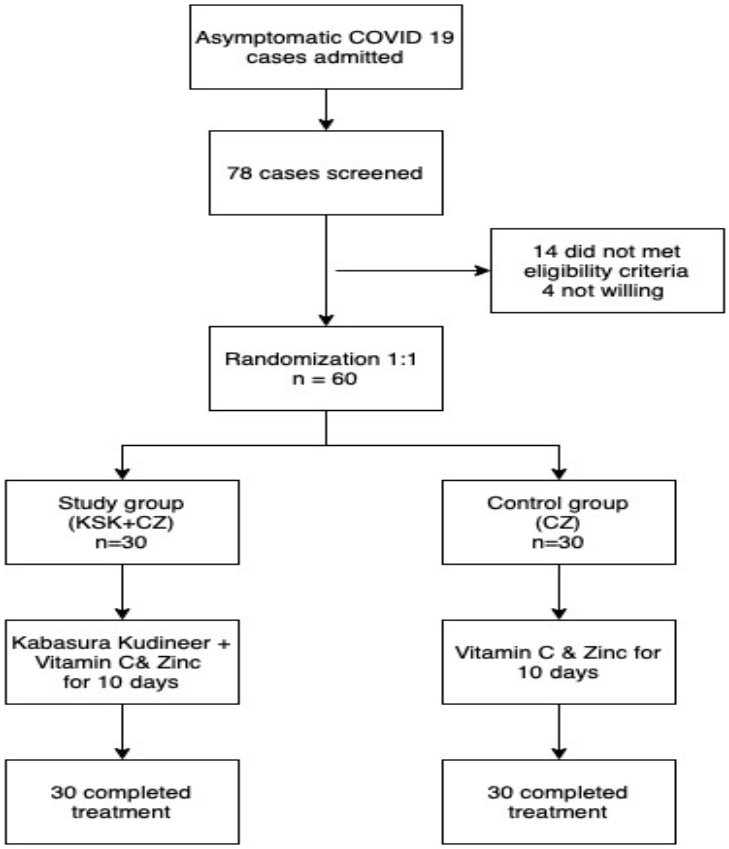

Fig.1: Trial flow chart

Table 1: Baseline characteristics of the study and control group

Characteristics		**Study group (n=30)**	**Control group (n=30)**
Age (in years) [Mean ± SD]		34.90 ± 10.69	34.60 ± 11.37
Male gender		21 (70%)	18 (60%)
Female gender		9 (30%)	12 (40%)
Education	Able to read and write	01	02
	Primary	01	01
	High school	10	08
	Higher secondary	02	05
	Degree	16	14
Exposure history	Contact with COVID-19 individual	27	25
	Don't know	03	05

Table 2: Investigational parameters of the study and control group at the baseline and end of the treatment.

Investigational parameters	**Study group**		**Control group**	
	Baseline	End line	Baseline	End line
Random blood sugar (80-140 mg/dL)	114.30± 19.18	119.56± 16.50	128.43 ± 22.26	107.00 ± 25.8
Sodium (136-145 mMol/L)	128.66± 6.18	136.20± 4.27	124.86 ± 7.47	135.90 ± 6.14
Potassium (3.5-5.1 mMol/L)	3.50± 0.68	4.33± 0.60	3.86 ± 0.57	4.40 ± 0.62
Haemoglobin 12-15 g/dL	11.87 ± 2.09	11.83 ± 1.41	11.67 ± 2.27	11.76 ± 1.59
Total RBC (4.2-5.4 x10^6/µl)	4.27 ± 0.74	5.33 ± 0.66	4.40 ± 0.72	5.27 ± 0.64
Total WBC (4-11x 10^3/µl)	8.33 ± 2.63	8.83 ± 1.17	8.33 ± 2.77	8.87 ± 1.25
Neutrophil count (40-75%)	62.30 ± 9.38	57.90 ± 6.13	71.33 ± 14.46	61.96 ± 8.89
Lymphocyte count (20-45%)	15.96 ± 4.18	23.83 ± 4.14	16.86 ± 3.60	20.93 ± 2.39

Investigational parameters	Study group		Control group	
	Baseline	End line	Baseline	End line
Mixedcell count (monocytes, basophils, and eosinophils)%	19.63 ± 5.54	18.86 ± 5.57	18.13 ± 5.94	19.03 ± 4.18
HCT 38-45%	34.52 ± 7.50	39.63 ± 5.59	35.90 ± 8.81	38.67 ± 5.97
MCV (80-100 fL)	79.73 ± 8.30	85.50 ± 5.37	73.70 ± 15.17	77.90 ± 10.58
MCH (27-32 pg)	28.87 ± 11.43	31.63 ± 4.42	28.07 ± 4.08	31.90 ± 3.45
MCHC (32- 36 g/dL)	32.90 ± 2.89	35.63 ± 2.78	33.77 ± 2.84	36.57 ± 2.30
PLT (150-450 x10^3/µl)	302.20 ± 86.48	302.37 ± 53.55	268.93 ± 79.57	289.40 ± 73.84

[Note: Data expressed in mean (SD)]

[Mixed cell count (monocytes, basophils, and eosinophils); HCT (Hematocrit); MCV (Mean Corpuscular Volume);MCH (Mean Corpuscular Hemoglobin); MCHC (Mean Corpuscular Hemoglobin Concentration); PLT (Platelet Count)]

Table 3: RT-PCR Cycle Threshold (ct) value of the study group compared to the control group at the end of the treatment with the baseline adjusted

Gene type	Group	SARS-CoV-2 Cyclic threshold value Mean (SD)	t- test				Adjusted baseline			
			Mean difference	95% CI		P value	Mean difference	95% CI		P value
				Lower	Upper			Lower	Upper	
ORF gene	study	36 (0.00)	1.033	0.048	2.019	0.04	1.038	0.043	2.032	0.041
	control	34.97(2.69)								
E gene	study	36(0.00)	1.00	0.05	1.95	0.039	0.996	0.041	1.951	0.041
	control	35(2.6)								
S gene	study	36 (0.00)	1.06	0.056	2.078	0.039	1.085	0.064	2.106	0.038
	control	34.93 (2.76)								

Table 4: Inflammatory markers, renal and liver function parameters of the study group compared to the control group at the end of the treatment with the baseline adjusted

Parameter	Group	Estimates	t- test				Adjusted baseline			
		Mean (SD)	Mean difference	95% CI		P value	Mean difference	95% CI		P value
				Lower	Upper			Lower	Upper	
Ferritin 13-232 ng/ml	study	186.77(58.83)	5.93	19.65	31.51	0.64	5.93	19.65	31.51	0.64
	control	192.70(37.93)								
D-dimer <0.5 µg/ml negative	study	0.03(0.18)	0.10	0.04	0.24	0.16	0.10	0.04	0.24	0.16
	control	0.13(0.34)								
CRP (<= 6 mg/L)	study	7.20(1.91)	0.96	0.16	2.09	0.09	0.967	0.163	2.09	0.09
	control	8.17(2.42)								
Blood urea (7.0-21 mg/dL)	study	28.63(7.69)	3.80	0.854	8.45	0.108	3.80	0.85	8.45	0.108
	control	24.83(10.14)								
Serum creatinine (0.9-1.3 mg/dL)	study	0.87(0.16)	0.016	0.08	0.12	0.75	0.01	0.09	0.12	0.75
	control	0.85(0.23)								
SGOT (8-40 U/L)	study	28.47(8.76)	3.33	3.48	10.15	0.33	3.33	3.48	10.15	0.33
	control	31.80(16.46)								
SGPT (8-45 U/L)	study	30.20(13.71)	1.30	8.07	10.67	0.78	1.30	8.07	10.67	0.78
	control	28.90(21.68)								
Alkaline phosphatase (53-128U/L)	study	106.50(28.99)	29.60	15.07	44.12	0.000	29.60	15.07	44.12	0.000
	control	76.90(27.18)								
Total Protein	study	7.59(0.66)	0.68	0.15	1.20	0.01	0.68	0.15	1.20	0.012
	control	6.91(1.27)								

(6.0-8.0 gm/dl)										
Albumin (3.5-5.2 gm/dl)	study	4.42(0.56)	0.62	0.30	0.94	0.000	0.62	0.30	0.94	0.000
	control	3.80(0.65)								
Globulin (2.3-3.6 gm/dl)	study	3.17(0.46)	0.03	0.32	0.39	0.83	0.07	0.27	0.43	0.66
	control	3.20(0.86)								
Total Bilirubin (0.1-1.2 mg/dL)	study	0.91(0.22)	0.35	0.25	0.46	0.000	0.35	0.25	0.46	0.000
	control	0.55(0.19)								
Direct Bilirubin (0.0- 0.3 mg/dL)	study	0.37(0.19)	0.18	0.10	0.26	0.000	0.18	0.10	0.26	0.000
	control	0.18(0.09)								
Indirect Bilirubin (0.1-1.0 mg/dL)	study	0.50(0.25)	0.13	0.02	0.24	0.017	0.13	0.02	0.24	0.01
	control	0.36(0.15)								

[CRP (C- Reactive protein); SGOT (Serum Glutamic Oxaloacetic Transaminase); SGPT (Serum Glutamic Pyruvic Transaminase);]

Table 5: Cytokine level of the study and control group at baseline and end line

	Study group (n=30)		**Control group (n=30)**	
Investigations (serum cytokine)	**Baseline**	**End line**	**Baseline**	**End line**
IL10 pg/ml	2.00 (1.75-2.00)	2.00 (1.00-4.00)	2.00 (2.00-4.00)	1.00 (0.00-1.00)
IL6 pg/ml	12.50 (5.00-34.75)	44.00 (7.50-106.75)	21.00 (3.75-134.25)	3.00 (2.00-14.50)
IL4 pg/ml	1.00 (0.00-1.00)	0.00 (0.00-0.00)	1.00 (0.00-1.00)	0.00 (0.00-0.00)
IL2 pg/ml	2.00 (1.89-2.00)	0.50 (0.00-1.00)	2.00 (1.00-2.00)	0.50 (0.00-1.00)
IL17 pg/ml	0.00 (0.00-3.00)	0.00 (0.00-0.00)	0.00 (0.00-2.00)	0.00 (0.00-0.00)
TNF alpha pg/ml	46.50 (12.25-83.50)	13.00 (3.00-36.00)	16.00 (3.75-104.00)	3.00 (0.00-15.00)
IFN gamma pg/ml	1.00 (1.00-1.00)	0.00 (0.00-0.00)	1.00 (0.75-1.00)	0.00 (0.00-0.00)

[Note: Data expressed as median (25th-75th percentile)]