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EVALUATING THE EFFICACY OF A TRADITIONAL SIDDHA FORMULATION AMONG COVID-INFECTED SYRIAN GOLDEN HAMSTER: AN IN VIVO STUDY

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ABSTRACT:

Background: The COVID-19 pandemic has overburdened the current healthcare system and highlighted the need to explore potent remedies in traditional medicine systems. **Objective:** The objective of the study is to establish the efficacy of Kabasura Kudineer in an invivo animal model for COVID-19: Syrian Golden Hamsters. **Methods:** A total of 19 female hamsters were grouped into mock control, placebo control, positive control (remdesivir) and test (KSK) and tested. They were first inoculated with the COVID-19 virus through the intranasal route. The test drug and placebo was administered through oral gavage while Remdesivir was administered through intraperitoneal route. The assessment parameters considered to evaluate the efficacy of the test drug were edema, inflammation and haemorrhages. The hamsters were monitored for any body weight reduction and negative reactions before being sacrificed to evaluate the safety of the intervention. Lung pathology and viral load were studied further for hamsters in each group. **Results:** Therapeutic use of Intraperitoneal injection of Siddha formulation KSK reduces the SARS-CoV-2 viral load and associated gross clinical parameters. Results showed a significant reduction of 65% in the viral load, no adverse events and an improvement in lung edema, haemorrhage, and congestion in comparison to the untreated group. **Conclusion:** We observed that the animals treated with KSK exhibited less severe pathology compared to the untreated infected group. No toxicity or adverse events were observed in the KSK group. This pre-clinical study supports the safety and efficacy of KSK.

Key words: *Kabasura Kudineer*, COVID 19, Syrian Golden Hamster, SARS-CoV-2

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged as a highly transmissible and pathogenic virus in late 2019, precipitating a global pandemic of acute respiratory disease known as COVID-19. [1] This pandemic, declared by the World Health Organization (WHO) on March 11, 2020, has not only posed a substantial threat to public health but also underscored the vulnerabilities within current healthcare systems. [2]

In light of the ongoing crisis, the exploration of potential remedies has extended to ancient healthcare systems, such as Ayurveda and Siddha medicine. Kabasura Kudineer, a polyherbal drug rooted in the Siddha system, has emerged as a promising candidate for the management of COVID-19. Originally a concoction, it was transformed into a more accessible tablet form by Sri Sri Tattva (Sriveda Sattva Pvt. Ltd.). Noteworthy for its immunomodulatory properties, this formulation inhibits viral propagation and spread by enhancing and restoring immunity.

Earlier studies, including those by Kiran et al. (2020) [3] and Koppala et al. (2020) [4], have highlighted the potential of Kabasura Kudineer in managing fevers and flu-like symptoms, further exploring its toxicity, anti-inflammatory, antipyretic, antibacterial, and antioxidant activities. The efficacy of this formulation has been documented in the treatment of swine flu and various viral diseases affecting the respiratory system, as indicated by Kumar et al. (2021). [5], [6]

The individual constituents of Kabasura Kudineer, each backed by earlier research, contribute to its efficacy. For instance, Piper longum (Pippali) [7] exhibits significant anti-inflammatory activity, while Zingiber officinale (Ginger) [8] boasts pharmacological, phytochemical, and toxicological properties. Tinospora cordifolia (Giloy) [9] demonstrates hepatoprotective and immunomodulatory effects, and Terminalia chebula (Haritaki), known as the 'King of Medicine,' exhibits diverse pharmacological activities. [10]

The rich composition also includes Syzygium aromaticum (Clove-Lavang), studied by Kaur and Kaushal (2019).[11] for its antibacterial, antioxidant, antifungal, and anti-inflammatory properties, and Tragia involucrate (Stinging nettle-Pitt Parni), documented for its relief in bronchitis and fever. [12] Vasaka (Adhatoda vasica), another key ingredient, has traditional uses in treating respiratory conditions.[13]

Despite the apparent efficacy in human clinical trials, these remedies lack a conventional drug development pathway. To establish their potential as potent solutions for future epidemics or viral pathogens, it is imperative to supplement human clinical trial data with rigorous animal studies. This study seeks to fill

this gap by investigating the therapeutic potential of Kabasura Kudineer, a Siddha polyherbal drug, in reducing viral load among SARS-CoV-2 infected Syrian golden hamsters—an essential step towards comprehensive pre-clinical evaluation.

METHODOLOGY

Study Design: 19 female Syrian golden hamsters, 6-8 weeks of age, were obtained from Tata Memorial Advanced Center for Treatment, Research & Education in Cancer (ACTREC), Bombay for the study. All the hamsters were intranasally inoculated with 100 µL of DMEM containing $1*10^6$ PFU/ml of the COVID-19 virus cell culture. The hamsters were divided into 4 groups namely, 4 in mock control; 5 in placebo intervention (1000mg/kg, oral gavage); 4 in Remdesivir intervention (15mg/kg through saline) and the remaining 6 hamsters received test intervention Kabasura Kudineer (1000mg/kg, oral gavage).

Ethical Statement: The study was approved by the institute animal ethics committee 2082/PO/Rc/S/19/CPCSEA.

Test material : Kabasura Kudineer preparation: Kabasura Kudineer is a polyherbal classical Siddha formulation containing 15 herbal drugs mixed in equal quantity.[14] (Table 1)They are *Chukku* (*Zingiber officinale), Thippali* (*Piper longum), Lavangam* (*Syzygium aromaticum*), *Cirukancoir ver* (*Tragia involvucrata*), *Akkirakaram ver* (*Anacyclus pyrethrum*), *Muliver* (*Hygrophila auriculata*), *Kadukkaithol* (*Terminalia chebula*), *Adathodei elai* (*Adhatoda vasica*), *Karpooravalli* (*Coleus amboinicus*), *Kostam* (*Saussurea lappa*), *Seenthil thandu* (*Tinospora cordifolia*), *Siruthekku* (*Clerodendrum serratum*), *Nilavembu* (*Andrographis paniculata*), *Vattathiruppi ver* (*Cissampelos pareira),* and *Korai kizhangu* (*Cyprus rotundus*). The material was procured from Sriveda Sattva Pvt. Ltd., Bangalore (Sri Sri Tattva). The drug was licensed by the Ministry of AYUSH, Govt. of India. It was supplied in powdered form and stored at 4°C until further use. All the herbs constituting *Kabasura Kudineer* were subjected to quality control analysis, and after a due approval process, ingredients were issued for production as fine powders. All the ingredients were blended with excipients, followed by granulation and drying.

The placebo tablets were made of starch (100%) which were tested for quality control and powdered form identical to *Kabasura Kudineer.*

Study Duration: The hamsters were acclimatized to BSL3 laboratory for 7 days' acclimatization, thereafter infected on day1 and received respective interventions for 3 days. On the 4^{th} day, the animals were euthanized by an overdose of isoflurane and sacrificed for viral load estimation and gross pathological examination.

Vero E6 cell preparation: A 96 well plate was coated with 200 µl containing approx. 30,000 Vero E6 cells in DMEM media with 10% FBS. The plate was incubated overnight (12–18 h) at 37° C to achieve a Vero E6 cell monolayer.

Methodology: After clinical examinations and gross pathological examinations of the hamster lungs for edema, inflammation and haemorrhages, the lungs were homogenized for about 15- 30 seconds using the Pro 200 homogenizer (Pro Scientific Inc., Monroe, CT, USA) in a final volume of 1 X 2 ml of sterile PBS in Wheaton Teflon-Glass tissue grinders (catalogue no. W012576). The homogenized tissue was centrifuged at 4000 rpm for 10 minutes to remove the debris, and the supernatant was collected. The volume of the supernatant was measured. 50 µl of samples (lung tissue) were serially diluted (10-fold) in DMEM, and each dilution was plated in a different well with the pre-formed Vero E6 cell monolayers and incubated for 1 h at 37 °C in a 5% CO2 incubator with shaking at 15 minute intervals. Again the cells were overlaid with 200 µL of DMEM: CMC and incubated for 3 days at 37 °C in 5% CO2. The cells were further washed with PBS and fixed by adding 200 µL of 4% formaldehyde for 30 minutes. Finally the cells were stained with 100 µL of 0.05% (w/v) crystal violet in 20% methanol and incubated for 30 minutes. The PFU per lung was calculated using the dilution factor and PFU per mL. Cell only control was used as a negative control.

Statistical Analysis: Lung viral loads were compared for the test items and control groups by a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison using GraphPad Prism software (Version 9).

RESULTS

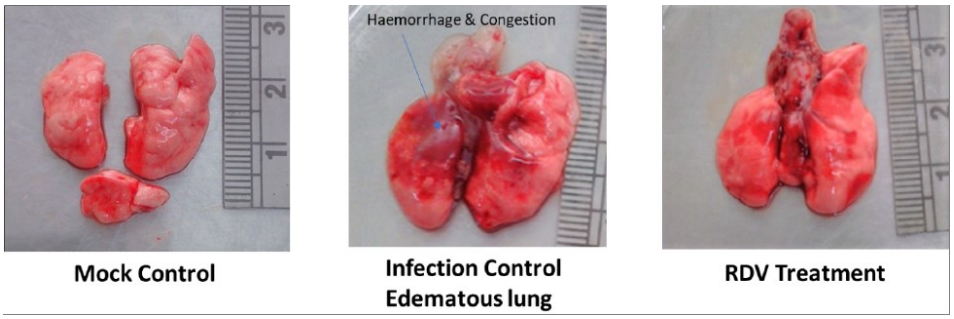
Table 1: Ingredients of *Kabasura Kudineer* tablet

Sr No	Ingredient	Parts	Proportion (mg)
1	*Zinziber Officinale*	Rhizome	33.33
2	*Piper longum*	Fruit	33.33
3	*Syzygium aromaticum*	Bark, bud	33.33
4	*Tragia involucrate*	Root	33.33
5	*Anacyclus Pyrethrum*	Root	33.33
6	*Hygrophilla auriculata*	Root	33.33
7	*Terminalia chebula*	Fruit	33.33

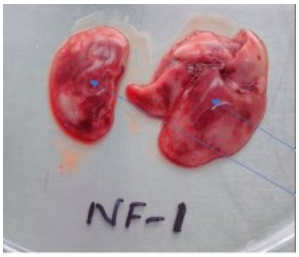
8	*Adathoda vasica*	Leaf	33.33
9	*Coleus ambonicus*	Leaf	33.33
10	*Saussurea lappa*	Root	33.33
11	*Tinospora cardifolia*	Stem	33.33
12	*Clerodendron serratum*	Root	33.33
13	*Andrographis paniculata*	Whole plant	33.33
14	*Cissampelos pareira*	Root	33.33
15	*Cyperus rotandus*	Rhizome	33.33

The results of the viral load examination and gross findings demonstrated an improvement in KSK treated hamster group.

Gross pathological observations of the untreated group demonstrated severe edema and inflammation in all lobes while the infected, untreated group also had diffused multi-focal hemorrhages and congestion. The infected remdesivir group showed mild edema and multi-focal congestion in the lungs. The KSK (NF-1) group showed improvement in lung edema, hemorrhage, and congestion in comparison to the untreated group.



Gross pathological examination



NF- 1

Figure 1: Representative gross images of the lungs from different study groups

The test item *Kabasura Kudineer* (NF-1) showed significant antiviral activity at 1000mg/kg BID dosing in comparison to the untreated control group. Compared to the infected and untreated groups, the reduction of lung viral load in the *Kabasura Kudineer* arm was 65%.

Table 2: Viral load reduction among different study groups

Group	Dpi	Animal No.	Number of plaques			PFU/ml	Log PFU/ml	Percentage Reduction
			1	2	Avg			
Mock Infection	4	1	0	0	0	0	0	0
		2	0	0	0	0	0	
		3	0	0	0	0	0	
		4	0	0	0	0	0	
Infection Control	5	5	19	18	18.5	3700000	6.57	
		6	18	14	16	3200000	6.51	
		7	22	25	23.5	4700000	6.67	
		8	16	18	17	3400000	6.53	
		9	21	19	20	4000000	6.60	
Remdesivir	4	10	5	8	6.5	130000	5.11	95.5
		11	12	10	11	220000	5.34	
		12	9	11	10	200000	5.30	
		13	6	9	7.5	150000	5.18	
Kabasura Kudineer	6	14	8	3	5.5	1100000	6.04	

		15	7	10	8.5	1700000	6.23	65
		16	6	3	4.5	900000	5.95	
		17	6	8	7	1400000	6.15	
		18	8	11	9.5	1900000	6.28	
		19	9	4	6.5	1300000	6.11	

Viral load estimation:

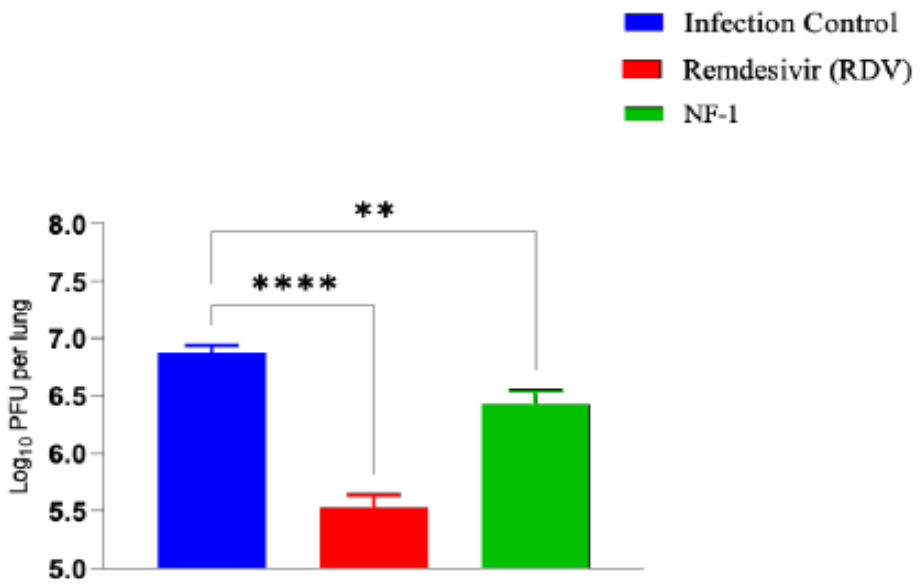


Figure 2: Log of Viral load reduction among the positive control (RDV) and *Kabasura Kudineer* (NF-1) group compared with the infection untreated group.

DISCUSSION

This pre-clinical research study investigates the safety and efficacy of the popular Siddha medicine *Kabasura Kudineer* in a Syrian golden hamster (animal) model. An earlier study suggested that features associated with SARS-CoV-2 infection in golden hamsters resemble those found in humans.[15] Another group evaluated the pathogenicity of SARS-CoV-2 isolates in hamsters after intranasal infection.[16] They found that the virus replicated efficiently in the respiratory tract and suggested that hamsters could serve as a useful mammalian model for COVID 19. These earlier studies support our methodology of

using the Syrian golden hamsters as an animal model to study the efficacy and safety of *Kabasura Kudineer*.

Traditional medicines have been used as therapeutic agents throughout human civilization. During the last century, with the advent of semi-synthetic and synthetic drugs, their popularity and use decreased. However, recently, the use of traditional medicines has increased once again because of growing reports of adverse side effects of synthetic drugs and the development of antibiotic resistance.[17,18] Traditional medicines are effective, but they are usually not supported by pre-clinical studies. Our study addresses that gap. The result indicates that *Kabasura Kudineer* is an efficacious option for the management of COVID-19. The study results demonstrated a 65% reduction in lung viral load in animals treated with *Kabasura Kudineer*. Moreover, lung pathology improved as compared to untreated controls. No side effects or toxicity were observed in animals.

Molecular docking studies of bioactive compounds from *Kabasura Kudineer* have confirmed their excellent binding efficiency with the spike protein of SARS-CoV-2.[19] A recent in vitro study on Vero E6 cell lines demonstrated the high antiviral efficacy of *Kabasura Kudineer* .[20] Chryseriol and luteolin from *Kabasura Kudineer* inhibit the ACE2 spike protein of SARS-CoV-2 .[21]

Kabasura Kudineer has shown great potential in clinical trials. Many clinical research studies on *Kabasura Kudineer* support its effectiveness as a therapeutic option along with standard treatment for mild and moderate patients of COVID-19. Results of several studies demonstrate that with the administration of KSK, clinical symptom resolution time was reduced by 3-6 days, cure rate was improved, disease progression was delayed, and the course of disease was shortened among COVID-19 patients.[22,23] *Kabasura Kudineer* increases immunity, acts as an immunomodulator, and can reinstate respiratory health.[24] In another clinical study, a reduction was observed in the viral load of SARS-CoV-2 reported at the end of treatment (10 days), as well as in the time taken to convert patients from symptomatic to asymptomatic based on their clinical symptoms during the 10 days of treatment.[25]

The results of pharmacological studies conducted on *Kabasura Kudineer* demonstrate it to be an effective drug in managing the symptoms of viral diseases affecting the respiratory system. The anti-inflammatory, antiviral, immunoprotective, and analgesic activities of its ingredients provide synergistic healing.[26]

Our pre-clinical study data supports the clinical observations. We observed that in the infection mock control of hamsters, intranasal

application of SARS-CoV-2 produced severe haemorrhage, congestion, oedema, and lung pathologies. However, in the *Kabasura Kudineer* treated group, the pathology severity was less. We observed that none of the hamsters in the *Kabasura Kudineer* group experienced any adverse events after the inoculation. This pre-clinical study supports the safety and efficacy of *Kabasura Kudineer*.

CONCLUSION

In the present study, *Kabasura Kudineer* was found to be safe and effective in an animal model of COVID-19. With the increasing incidence of side effects from synthetic drugs, it is important to search for safe and effective medicines for COVID-19 management. The hamsters treated with *Kabasura Kudineer* demonstrated a reduction in viral load, and their pathological severity was reduced in comparison to untreated animals. The results of our pre-clinical study support the safety and efficacy of *Kabasura Kudineer*, as well as its wider use in clinical settings as a treatment for COVID-19.

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Conflict of interest

The test resources were provided by Sri Sri Tattva, / Sriveda Sattva Pvt. Ltd., India. Dr Ravi Reddy is the chief scientific officer of Sriveda Sattva Pvt. Ltd.; In addition, Dr. Hari Venkatesh is the research and management head at Sriveda Sattva Pvt. Ltd. Besides providing the tablets, Sriveda Sattva Pvt. Ltd. was not involved in any aspect of this study. All the other authors have no conflicts of interest to declare.

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Ethical statement

Data availability

The raw data that supports the findings will be available upon request as supplementary files .

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