# *In vivo* efficacy and safety of Siddha medicine Kabasura Kudineer among COVID 19 infected Syrian golden hamsters.

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**Research Article**

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# Abstract

Background: The COVID-19 pandemic has overburdened current healthcare system and highlighted the need to explore potent remedies in Traditional medicine systems. Kabasura Kudineer (KSK), a poly herbal Siddha medicine, has shown great potential in treating COVID-19.

Objective: The objective of the study is to explore the safety and efficacy of Kabasura Kudineer in a preclinical model for COVID-19: Syrian Golden Hamsters.

Methods: This research study investigates the *in vivo* efficacy and safety of the well-known antiviral Siddha medicine KSK as a powdered tablet on COVID-19 infected Syrian golden hamsters. A total of 19 female hamsters were infected with the virus cell culture through intranasal route. 4 out of 19 animals were mock controls, 5 were infection controls, 4 were treated with remdesivir and acted as positive controls and remaining 6 were treated with KSK. The hamsters were observed for any adverse events, followed by their sacrifice on day 4 after inoculation with the virus. The lung pathology and viral load was studied for each hamster.

Results: Therapeutic use of intraperitoneal instillation of Siddha formulation KSK reduces SARS-CoV-2 viral load and associated gross clinical parameters. Results showed significant reduction of 65% in the viral load for the KSK arm as compared to the infection control.

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.

Study Registration: FNDR's Institutional Animal Ethics Committee (IAEC), Registration Number 2082/PO/Rc/S/19/CPCSEA


# Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible and pathogenic virus that emerged in late 2019. It's spread has led a pandemic of acute respiratory disease, namely COVID-19, that has threatened public health and safety across the world (Ben et al, 2021). The World Health Organization (WHO) declared the novel coronavirus (COVID-19) outbreak a global pandemic on March 11, 2020 (WHO DG media briefing, 2020).

The pandemic has highlighted the gaps in current healthcare systems and emphasized the dire need to look for potent remedies in ancient healthcare systems like Ayurveda and Siddha medicine. Kabasura Kudineer is a popular polyherbal drug from the Siddha system of medicine, which has shown great potential for the management of COVID-19. It is typically used as a concoction. To increase the ease of consumption, it was first converted to a tablet form by Sri Sri Tattva (Sriveda Sattva Pvt. Ltd). Many ingredients of Kabasura Kudineer are immunomodulators and inhibit the propagation and spread of virus

by enhancing and restoring immunity. Kiran et al, 2020 has mentioned its use for management of phlegmatic fevers and flu-like symptoms.

Koppala et al, 2020 studied the toxicity, anti-inflammatory, antipyretic, antibacterial and antioxidant activities of Kabasura Kudineer. Earlier study by Thillaivanan et al, 2015; researched its use for treating swine flu. According to Kumar et al, 2021; Kabasura Kudineer is effective in managing the symptoms of viral diseases affecting the respiratory system, due to its anti-inflammatory, antiviral, immunoprotective, antipyretic, and analgesic properties.

Individual constituents of Kabasura Kudineer have shown several benefits in earlier research studies; Kumar et al, 2009 demonstrated the significant anti-inflammatory activity of *Piper longum.* (Pippali) Pharmacological, phytochemical and toxicological properties of *Zingiber officinale* (Ginger) have been documented by Chang et al, 2013. Hepatoprotective and immunomodulatory effects of *Tinospora codifolia* (Giloy) were established by Bishayi et al, 2002. *Terminalia chebula,* (Haritaki) another constituent of KSK, also known as the 'King of Medicine' has been shown to possess multiple pharmacological and medicinal activities, including antioxidant, antimicrobial, antidiabetic, hepatoprotective, anti-inflammatory, antiarthritic and wound healing (Bag et al, 2013) properties. Kaur and Kaushal, 2019 studied the antibacterial, antioxidant, antifungal, and anti-inflammatory activity of *Syzygium aromaticum,* (Clove- Lavang) which is also found in *KSK. Tragia involucrate* (Stinging nettle-Pitt Parni) found in KSK has been documented to be useful in relieving bronchitis and fever (Dhara et al, 2000). Vasaka (*Adhatoda vasica)* , another ingredient of KSK is used for treating cold, cough, chronic bronchitis and asthma, especially where the sputum is thick and sticky (Hossain and Hoq, 2016).

Due to its potent ingredients, KSK has been gaining popularity in the treatment and management of COVID-19. Several human clinical trials have been conducted or are underway that investigate the efficacy of KSK in COVID-19 patients. At the time of publication, there are 11 clinical trials registered in CTRI, India that use KSK as an intervention for management of COVID-19. Although effective in humans, these drugs do not move through a typical drug development pathway. In order for these drugs to emerge as a potent solution for COVID-19, it is necessary to supplement the human clinical trial data with animal studies that measure safety and efficacy in a pre-clinical setting.

Even though KSK is gaining popularity and emerging as a potential therapeutic option for COVID-19, to the best of our knowledge, there is not yet a single animal study that measures efficacy and safety of this drug in a pre-clinical setting. To bridge this gap, this research study investigates the *in vivo* efficacy and safety of the well-known antiviral Siddha Drug Kabasura Kudineer as a powdered tablet on COVID-19 infected Syrian hamsters.

# Objective

To evaluate the in vivo therapeutic efficacy of Kabasura Kudineer, a Siddha polyherbal drug in SARS CoV-2 infected Syrian golden hamsters (*Mesocricetus auratus*) model.

# Methodology

Formulation for in vivo studies

1.1 Test material

Kabasura Kudineer preparation: Kabasura Kudineer is a polyherbal formulation containing 15 herbal drugs mixed in equal quantity. 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 a powdered form and stored at 4$^{o}$C until further use. All the herbs constituting Kabasura Kudineer were subjected to quality control analysis and after due approval process, ingredients were issued for production as fine powders. All the ingredients were blended with excipients followed by granulation and drying.

The animal study was conducted at Foundation for Neglected Disease Research (FNDR) in the BSL-3 laboratory. All the ethical guidelines with respect to the animal study were met in accordance.

1.2 Test Item Preparation

Stock solution of the test item Kabasura Kudineer was prepared in saline.

1.3 Animal Model: Syrian Golden Hamsters

6-8 weeks old female Syrian golden hamsters (Tata Memorial Advanced Center for Treatment, Research & Education in Cancer (ACTREC), (65/PO/ReBiBt/S/99/CPCSEA)) were included in the study. Body weight examination and other general veterinary examinations of hamsters were performed at the time of enrollment. Only healthy animals weighing around 80-100 grams were included in the study.

A total of 19 hamsters were enrolled, each group consisting of 4-6 animals.

1.4 Animal care:

All the animals were kept and familiarized in Individually Ventilated Cages (Citizen Industries, CRB-48-SS, V7E). The room conditions were maintained at 18$^{o}$C-25$^{o}$C temperature, 30%-70% humidity, 12-hour light

and 12-hour dark. The animals were identified by body markings and therefore maintained in different groups. All the hamsters were provided with Feed and RO water *ad libitum.*

1.5 Animal ethics statement

The study plan for measuring the antiviral activity in COVID 19 infection model in hamsters was recommended by FNDR's Institutional Animal Ethics Committee (IAEC), Registration Number 2082/PO/Rc/S/19/CPCSEA, on 21st December 2021 through Form-B proposal number FNDRFB-045. It was further approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. All ethical practices, as laid down in the CPCSEA guidelines for animal care, were followed during the conduct of the study. Additionally, the procedures used in this study plan were designed to conform to the accepted practices and to minimize or avoid risk of causing pain, distress or discomfort to the animals.

1.6 Viral Cell culture for infection:

The SARS-CoV-2 viral isolate was obtained by BEI resources managed by ATCC. Isolate USA-WA1/2020 was isolated from an oropharyngeal swab from a patient with a respiratory illness who had recently returned from travel to the affected region of China and developed clinical disease (COVID-19) in January 2020 in Washington, USA.

1.7 Study Duration

7 days' acclimatization in BSL3, 1-day infection, 3 days dosing, 1-day sacrifice and plating, 7 days PFU counts.

1.8 Study Design

19 female Syrian golden hamsters, 6-8 weeks in age, were obtained for the study. Among the 19 hamsters, 4 hamsters were not infected with virus and were labelled as mock control, 5 hamsters were infected with virus and received placebo intervention and were labelled as disease control, 4 other hamsters were infected with virus and received remdesivir intervention and were labelled as positive control while remaining 6 hamsters were infected with virus and received test intervention Kabasura Kudineer and were labelled as test.

Hamsters were anesthetized with ketamine (150mg/kg) and xylazine (10mg/kg) via intraperitoneal route and inoculated with the virus intranasally with 100µL of DMEM containing 1× 106 PFU/ml. Hence, the total concentration of virus administered per hamster was 1× 105 PFU. The hamsters received respective intervention 24 hours post infection.

The respective intervention was administered via intraperitoneal route. Positive control animals were provided Remdesiver at 15mg/kg, for 3 days daily post infection. The test animals were provided Kabasura Kudineer intervention at 1000mg/kg in saline for 3 days daily post infection.

On the 4th day, the animals were euthanized by an over dosage of Isoflurane and sacrificed for viral load estimation and gross pathological examination.

1.9 Viral Load estimation

After sacrificing the hamsters, the following procedure was carried out for viral load estimation and gross pathological examination.

Sample Preparation: The whole lung was aseptically removed from the sacrificed animal. Changes in the body weight before and after clinical administration were noted. After gross pathological examination, the lung was homogenized for about 15- 30 seconds using Pro 200 homogenizer (Pro Scientific Inc. Monroe, CT. USA) in a final volume of 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. Volume of the supernatant was measured.

Vero E6 cells preparation: A 96 well plate was coated with 200ul 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.

Sample plating: 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% $CO_2$ incubator with shaking at 15 minute intervals.

Overlay: After 1-hour incubation, the samples were removed from the well. The cell monolayer was again overlaid with 200 µL of DMEM: CMC and incubated for 3 days at 37°C in 5% $CO_2$. DMEM: CMC was prepared by mixing equal volume of DMEM (2x) and 2% carboxymethylcellulose.

Fixing: After the 3-day incubation, the DMEM-CMC overlay was gently removed with a pipette, the cells were washed twice with PBS and then fixed by adding 200 µL of 4% formaldehyde to each well. The plate was incubated at room temperature for 30 minutes, after which formaldehyde was removed from the wells and dispensed in an appropriate hazardous waste container.

Staining: 100 µL of 0.05% (w/v) crystal violet in 20 % methanol was added to each well and the plate was incubated for 30 minutes. Crystal violet was removed with a pipette and cells were washed twice with distilled water or until excess crystal violet was removed, and plaques are easily visualized.

Counting: The plaques (PFU) were counted for the dilution at which clear readable counts were noticed and were recorded as PFU per ml. The PFU per lung was calculated using the dilution factor and PFU per ml. Cell only control was used as a negative control.

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

# Results

Therapeutic use of intraperitoneal instillation of Siddha formulation Kabasura Kudineer reduces SARS-CoV-2 viral load and associated gross clinical parameters.

Gross pathological examination

The test item KSK (NF-1) showed a significant antiviral activity at 1000mg/kg BID dosing in comparison to the untreated control group. Gross pathological observations demonstrated normal hamster lungs in the non-infected group. The infected untreated group demonstrated severe edema and inflammation in all lobes. The infected untreated group also had diffused multi-focal hemorrhages and congestion. The infected remdesivir group showed mild edema and multi-focal congestions in the lungs. The KSK (NF-1) group showed improvement in lung edema, hemorrhage and congestion in comparison to the untreated group.

Viral load estimation

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

Table 1
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 | 65 |
| | | 15 | 7 | 10 | 8.5 | 1700000 | 6.23 | |
| | | 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 | |

# Discussion

This pre-clinical research study investigates safety and efficacy of popular siddha medicine Kabasura Kudineer in Syrian golden hamster (animal) model. Earlier study by Sia et al, 2020 suggested that features associated with SARS-CoV-2 infection in golden hamsters resemble those found in humans. Imai et al, 2020 evaluated the pathogenicity of SARS-CoV-2 isolates in hamsters after intranasal infection. They found that the virus replicated efficiently in the respiratory tract and suggested that hamsters could serve as 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 the human civilization. During last century, with 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 development of antibiotic resistance (Mohammed, 2012; Capodice and Chubak 2021). 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 management of COVID-19. The study results demonstrated a 65% reduction in lung viral load in animals treated with Kabasura Kudineer. Moreover, the lung pathology improved as compared to untreated controls. No side effects or toxicity was observed in animals.

Molecular docking studies of bioactive compounds from Kabasura Kudineer have confirmed its excellent binding efficiency with spike protein of SARS-CoV-2 (Maideen,2021). A recent in vitro study on Vero E6 cell lines demonstrated high antiviral efficacy of Kabasura Kudineer ( Kanchibhotla et al, 2021). Chryseriol and Luteolin from Kabasura Kudineer inhibit ACE2 spike protein of SARS-CoV-2 (Kiran et al, 2020).

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 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 (Bala D, 2021; Natarajan et al, 2020). Kabasura Kudineer increases immunity, acts as an immunomodulator and can reinstate the respiratory health (Ramya et al, 2021). 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 (Srivastava et al, 2021).

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 (Kumar et al, 2021).

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 hemorrhage, congestion, edema 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 with 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 pathology 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 support its wider use in clinical settings as a treatment for COVID-19.

# Declarations


### Acknowledgements

We would like to acknowledge the Foundation for Neglected Disease Research (FNDR) for conducting the experiment in their biosafety lab 3 facility. We would also like to acknowledge Dr. Somya Ramrakhyani for language edits.


### Author Contributions

Conceptualization: DK (Divya Kanchibhotla)

Methodology: DK,

Validation: DK.

Formal analysis: DK.

Resources: RKR, VKH. (Reddy M. Ravi Kumar, Venkatesh Hari K. R)

Data curation: JP(Jeetu Pathak)

Writing – Original Draft: JP

Writing – Review & Editing: DK,

Supervision: DK.

Project administration:  JP

Funding acquisition: DK.

### 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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We would like to thank Cooper Family Foundation, Australia for providing us the funding for this *in vivo* study


### Ethical statement

The study was conducted at The Foundation for Neglected Disease Research (FNDR) in their biosafety lab 3 facility. The study was reviewed and approved by FNDR's Institutional Animal Ethics Committee (IAEC), Registration Number 2082/PO/Rc/S/19/CPCSEA, on 21st December 2021 through Form-B proposal number FNDRFB-045 and further the same was approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

### Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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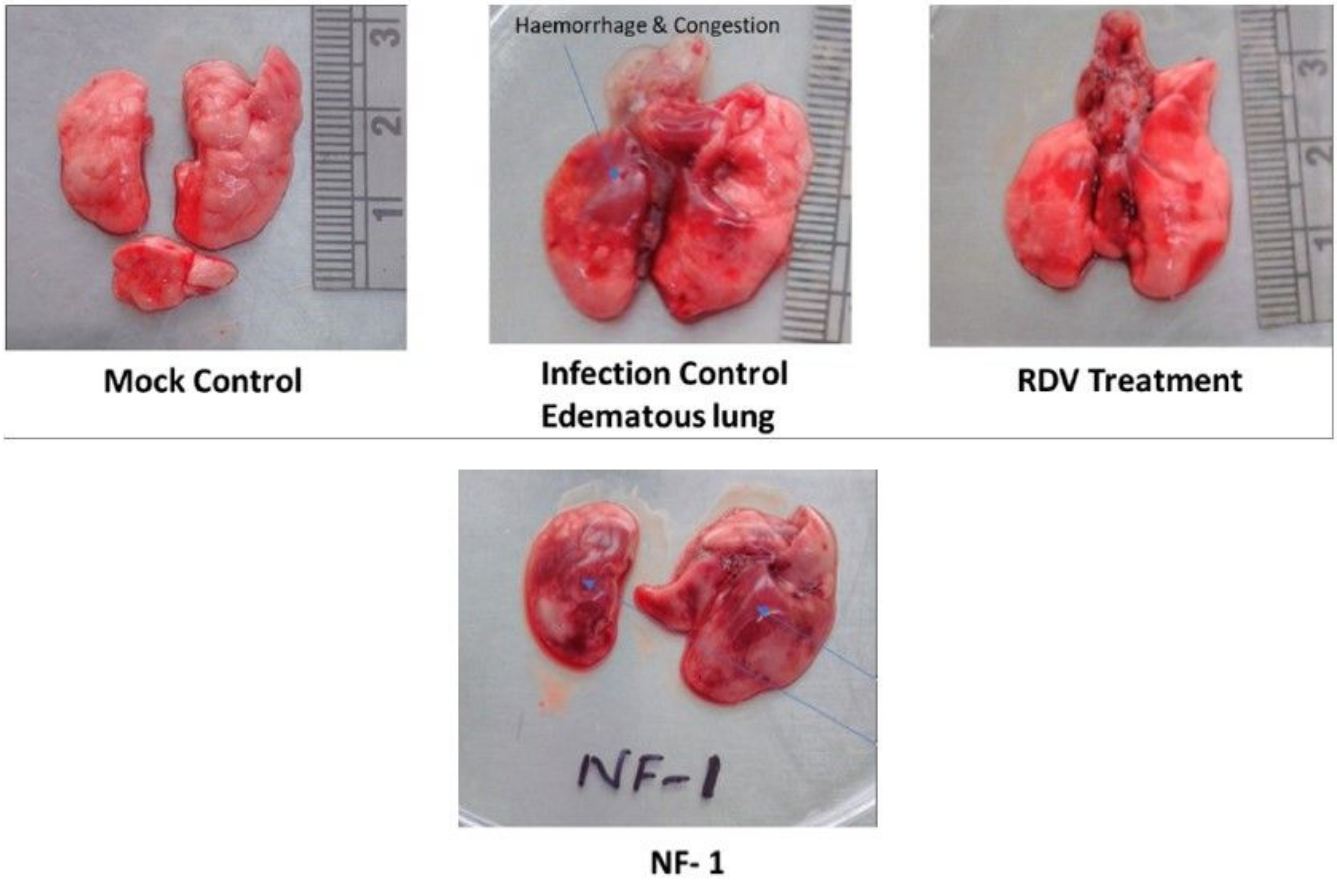
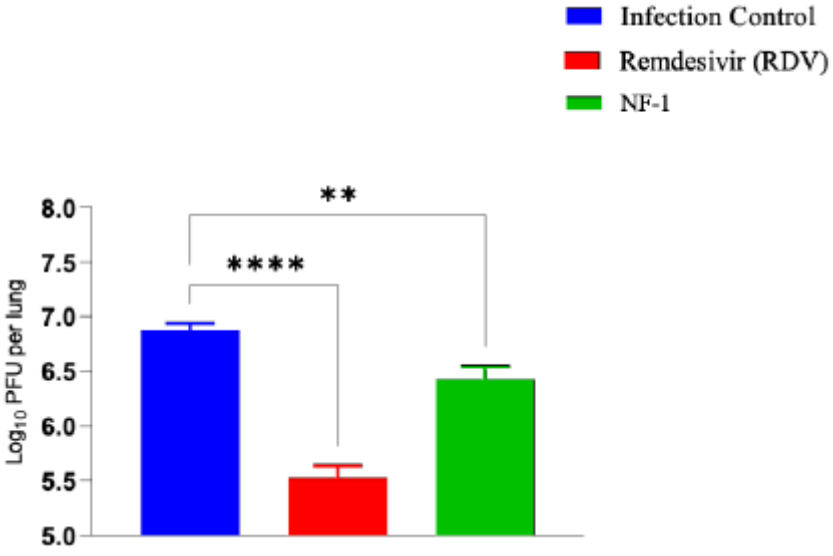
# Figures




## Figure 1

Representative gross images of the lungs from different study groups




## Figure 2

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