SCIENTIFIC VALIDATION OF SIDDHA HERBAL FORMULATION DEVA CHOORANAM AGAINST NOVEL CORONAVIRUS (2019-nCoV/COVID-19)

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ABSTRACT

The outbreak of respiratory illness leading to mortality brought about by a novel (new) coronavirus (named “2019-nCoV”) first detected in Wuhan City, Hubei Province, China still despite every efforts continue to extend. With the virus reportedly spreading from person-to-person in many parts of that country, the Chinese health officials have reported thousands of infections with 2019-nCoV in China. About 2000 instances of 2019-nCoV disease have been affirmed as of January 2020 and keeps on broadening all around. With technological advances and more prominent duty from governments around the globe including the WHO to fund and look into the rising infection, the endeavors to think of an antibody for vaccination or a novel medication to battle this destructive ailment is still at an early stage. In this scenario there has been emerging claims in social media around the globe regarding several alternative measures with no verifiable scientific evidence to support their actual benefits. While traditional medicine like Siddha or Ayurveda is a treasure of India, the safety and effectiveness of these medicines are still under debate among both adherents and skeptics. Though many of the remedies have been in use for hundreds of years, critics argue the claims of therapeutic efficacy of traditional medicine. In this aspect through this article we have laid a ground work on scientifically validating a polyherbal Siddha formulation Deva chooranam (DC) with proven preclinical safety and efficacy against HIV to have possible beneficial effects for the prevention and management of 2019-nCoV infection.

INTRODUCTION

Coronaviruses are large, enveloped, positive-strand RNA viruses that are named for the crown-like spikes on their surface. They can be divided into 4 genera: alpha, beta, delta, and gamma, of which alpha and beta CoVs are known to infect humans. Rarely, animal coronaviruses can infect people and then spread between people such as with MERS, SARS, and now with 2019-nCoV.1 The seven coronaviruses that can infect people are:

1. 229E (alpha coronavirus)
2. NL63 (alpha coronavirus)
3. OC43 (beta coronavirus)
4. HKU1 (beta coronavirus)
5. MERS-CoV (the beta coronavirus that causes Middle East Respiratory Syndrome, or MERS)
6. SARS-CoV (the beta coronavirus that causes severe acute respiratory syndrome, or SARS)
7. 2019 Novel Coronavirus (2019-nCoV)

Sometimes coronaviruses that infect animals can evolve and make humans sick and become a new human coronavirus.2 Three recent examples of this are 2019-nCoV, SARS-CoV, and MERS-CoV. 2019-nCoV is a beta coronavirus, similar to MERS and SARS, all of which have their sources in bats. Early on, a considerable lot of the patients in the flare-up of respiratory infections brought about by 2019-nCoV in Wuhan, China had some connection to a large seafood and live animal market, recommending animal to human spread. Afterward, a budding number of patients who supposedly didn't have exposure to animal market, also showed individual to individual spread. The Chinese authorities report that constant individual to individual spread is happening among Chinese
community and has also been accounted for cases outside China, including the United States and few other nations. Furthermore, asymptomatic spread of the infection have been reported. Towards the beginning of January, the flare-up started to heighten quickly with several cases presently confirmed with the presence of a few household clusters.  

**De and Siddha Pathologic Basis of Diseases**

DC is a combination of three medicinal herbs, Cedrus deodara (Devardara), *Alpinia galanga* (Arathai), *Cinnamomum tamala* (Lavanga pathiri). Each of these herbs has promulgated their hundred-proof medicinal properties by redressing man from some of the ailments and diseases which have proved to deadweight burden to mankind for ages. According to Siddha concept, the physiological function in the body is mediated by three Humours *Vatham, Pitham, and Kabam* which are said to maintain the integrity and function of our body. *Vatham* is formed by the basic elements space and air. *Pitham* is formed by fire and *Kabam* is formed by earth and water. The Siddha philosophy relies on the concept that any alteration in these humours caused by diet and lifestyle can result in dosham or diseases. The choice of these three herbs mentioned in *Agathiyr gunavagadam* of classical Siddha literature is based on the indications mentioned such as Chronic fever, diarrhea, dysentery, oral ulcers, respiratory ailments, skin diseases and tumours that can be found in the co existing clinical conditions of HIV infected individuals.  

<table>
<thead>
<tr>
<th>S.No</th>
<th>Botanical Name</th>
<th>Tamil Name</th>
<th>Suvaith (Taste)</th>
<th>Action on humours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Alpinia galanga</em></td>
<td>Sitrathai</td>
<td>Karpu (Acrid)</td>
<td>Pacifies vitiated kabam</td>
</tr>
<tr>
<td>2.</td>
<td>Cedrus deodara</td>
<td>Thevathaaar</td>
<td>Kaippu (Bitter)</td>
<td>Pacifies vitiated pitham and kapham</td>
</tr>
<tr>
<td>3.</td>
<td><em>Cinnamomum tamala</em></td>
<td>Lavanga pathiri</td>
<td>Karpu (Acrid)</td>
<td>Pacifies vitiated kabam</td>
</tr>
</tbody>
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The clinical symptoms of patients of 2019-nCoV infection were cough, myalgia or fatigue, sputum production, headache and diarrhoea acute respiratory distress syndrome, acute cardiac injury and secondary infection. MERS shares many clinical features with SARS such as severe atypical pneumonia, yet key differences are evident. Patients with MERS have prominent gastrointestinal symptoms and often acute kidney failure, likely explained by the binding of the MERS-CoV S glycoprotein to dipeptidyl peptidase 4 (DPP4), which is present in the lower airway as well as the gastrointestinal tract and kidney. The Siddha pathologic basis of these clinical symptoms are almost similar to that of HIV and has a common Siddha pathology of altered Kapha and Pitha humour (Table1).  

**Scientific Data on De against HIV**

Upon considering the traditional literature evidences of the ingredients of DC and analysing them in the light of scientific researches, it can be found that the bitter taste of Cedrus deodara pacifies both the vitiated humors (pitham and Kapham) and also has immunomodulatory property. The plant *Alpinia galanga* is scientifically reported to inhibit the the replication of Human Immunodeficiency Virus. Both *Alpinia galanga* and *Cinnamomum tamala* possess acrid taste (Pacifying kabam) has been found to have potent immunomodulatory activity. The plant *A. galanga* has a rich treasury of therapeutic phytochemicals. It has an abundance of phenolic compounds like phenolic acids and flavonoids. The antioxidant activity of the plant was first reported by Padma S. Vankar et al., in which the methanolic extract of *Alpinia galanga* showed better activity compared to other Zingiberaceae counterparts.  

Ying and Baon isolated the 19S-19- Acetoxychavicol acetate and first reported that the compound inhibited the replication of Human immune deficiency virus by blocking the transport of the Regulatory HIV-I protein (Rev). Bendjeddion et al., demonstrated the immunostimulating activity of polysaccharide extracts of *Alpinia galanga* on the reticulo-endothelial system (RES) and peritoneal exudate cells (PEC) and spleen cells of mice. The study by Chaurasia JK et al.,clearly portrays that non-polar hexane fraction of leaves of *Cinnamomum tamala* possesses immunosuppressive property, which is mediated through modulation of innate immunity. Literature have revealed the various pharmacological activities of the tree Cedrus deodara and its oil like antibacterial, insecticidal activity, Molluscidal activity, antitubercular activity, anxiolytic and anticonvulsant activity, neuroleptic activity, antidiabetic activity, antioxidative activity, antimalarial activity and cytotoxic activity.  

**Molecular Docking Studies of DC Against HIV-1 RT**

Molecular docking studies were performed with the lead components of DC, namely, Eugenol, Kaempferol, Atlantone, Apigenin and Pinene has revealed to have maximum interactions when compared to that of the standard Nevirapine and revealed RT enzyme inhibition activity through Molecular Docking methods. All the leads have indicated marked interactions with the RT as when compared with Nevirapine a standard RT inhibiting anti-HIV drug. Among them, the compound Eugenol indicated Eugenol has specific interactions number of associations with aminoacid Tyrosine, Tryptophan and Phenylalanine. The present atomic docking study results uncover that Eugenol has explicit interactions with aminoacid deposits at 181TYR, 182TYR, 227 PHE, 318TYR 318TYR including the highly conserved residue TRP229 like that of the standard Nevirapine. The other two phytocompounds Kaempferol and Atlantone demonstrated comparative collaborations of official with aminoacid deposits when contrasted and standard Nevirapine aside from 229 TRP. In a synergetic mode the lead compounds of DC may check opportunistic infections and may have considerable benefit in the management of AIDS.  

**Coronaviruses and Structural Similarity of 2019-Ncov With HIV and SARS-CoV**

The most prominent feature of coronaviruses is the club-shape spike projections emanating from the surface of the virion. These spikes are a defining feature of the virion and give them the appearance of a solar corona, prompting the name, coronaviruses. Coronavirus virus particles contain four main structural proteins. These are the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins, all of which are encoded within the 3′ end of the viral genome.
The S-protein/receptor interaction is the primary determinant for a coronavirus to infect a host species and also governs the tissue tropism of the virus. Many coronaviruses utilize peptidases as their cellular receptor though unclear. All viruses in the *Nidovirales* order are enveloped, non-segmented positive-sense RNA viruses. Other common features within the *Nidovirales* order include a highly conserved genomic organization, with a large replicase gene preceding structural and accessory genes, expression of many nonstructural genes by ribosomal frame shifting, several unique or unusual enzymatic activities encoded within the large replicase-transcriptase polyprotein and expression of downstream genes by synthesis of 3’ nested sub-genomic mRNAs. The major differences within the Nidovirus families are in the number, type, and sizes of the structural proteins. These differences cause significant alterations in the structure and morphology of the nucleocapsids and virions.\(^\text{19}\) Dr. Eric Feigl-Ding, a Chinese-American epidemiologist and public health scientist, referenced the paper explaining the pathology of the virus and the possible misinformation about the epidemic provided by the Chinese government. He emphasized that the researchers have found 4 insertions in the spike glycoprotein (S) which are unique to the 2019-nCoV and are not present in other coronaviruses,” the report’s Abstract section states. “Importantly, amino acid residues in all the 4 inserts have identity or similarity to those in the HIV-1 gp120.” The report also mentions that “interestingly, despite the inserts being discontinuous on the primary amino acid sequence, 3D-modelling of the 2019-nCoV suggests that they converge to constitute the receptor binding site.”\(^\text{20,21}\) Most promisingly, two drugs given together to treat HIV – called lopinavir and ritonavir – are already approved for human use, and in small trials they seemed to reduce disease severity and fatalities of coronaviruses.\(^\text{22}\)

A recent research by Yamamoto N et al., demonstrated that HIV-1 protease inhibitor, nelfinavir, firmly hindered replication of the SARS coronavirus (SARS-CoV). Nelfinavir restrained the cytopathic impact provoked by SARS-CoV infection. Expression of viral antigens was much lower in infected cells treated with nelfinavir than in untreated infected cells. Quantitative RT-PCR investigation demonstrated that nelfinavir could diminish the generation of virions from Vero cells. Experiments with addition of several drugs at various times revealed that nelfinavir exerted its effect not at the entry step, but at the post-entry step of SARS-CoV infection. Their outcomes recommend that nelfinavir ought to be analyzed clinically for the treatment of SARS and has a possibility to be a potent lead compound for drug designing against SARS-CoV.\(^\text{23}\)

Owing to these contributions of Nelfinavir, it was predicted to be a potential inhibitor of 2019-nCov main protease by an integrative approach combining homology modelling, molecular docking and binding free energy calculation. A recent study conducted by Zhijian Xu in china built 11 homology models of 2019-nCov Mpro and docked 1903 approved small molecule drugs to the 2GTB model. Based on the docking score and the 3D similarity of binding mode to 39 known Mpro binders, 15 drugs were selected for further evaluation. The results of the study suggested that nelfinavir might be active against 2019-nCov Mpro. In addition, pitavastatin, perampanel, and praziquantel might also have moderate activities against 2019-nCoV.\(^\text{24}\)

**Significance of de for the Prevention and Management of 2019-ncov**

Presently there exist two ways of treating viral infections.

1. To find small molecules that stop viruses replicating by interfering with viral proteins.
2. The second way is to use the same weapons that our bodies use: antibodies. Antibodies are large proteins that bind to viruses and trigger their destruction.

Upon analysing the first option of small molecules, 99 per cent of potential small-molecule drugs fail. So developing new antivirals from scratch could take years. Secondly, antibodies are less likely to cause side effects than small-molecule drugs, because they bind more specifically to viruses whereas small-molecule drugs tend to stick to lots of other things as well. This means we should be able to find safe and effective antibodies against the 2019 coronavirus very quickly.\(^\text{25}\) On evaluating the herbal ingredients of DC and its molecular docking studies against HIV, in the limelight of recent invention on the structural similarity of 2019-CoV with HIV and the advantages of anti-retroviral drugs against 2019-CoV, it can be well understood about the possible therapeutic benefits of *Siddha* herbal drug DC against the prevention and management 2019-CoV possibly by immunomodulatory action or by inhibiting the viral replication(Fig-1). Recent researches on herbs reveal that the therapeutic effects of plant extracts have been suggested to be due to their influence on the immune system of the human body.\(^\text{26}\) The innate immune system is the main line of defense against microbial infection and consists of type I interferons (IFNs) and pro-inflammatory cytokines.\(^\text{27}\) Type I IFNs, α and β, are synchronized by IFN regulatory factor 7 (IRF-7), IRF-3, NF-kB and a number of intracellular signaling molecules, which are activated by germline-encoded pattern recognition receptors that identifies the molecular pattern in particular to microorganisms.\(^\text{28,29}\) There are sufficient reports revealing immunomodulatory properties of many plant extracts mediated through induction and release of pro-inflammatory cytokines, IL-6 and IL-12. IL-12, produced by activated monocytes/macrophages and dendritic cells, stimulate cell-mediated immunity to release IFN-γ, promote Th1 responses, and enhance CD8+ cytotoxic T cell activity, thereby playing a crucial role in controlling viral replication.\(^\text{30}\) Therefore, those plant extracts or phytoconstituents, which exhibit potential immune-stimulating effects may be useful in phagocytic enhancement, necessary for attenuating viral load and preventing infection spread.\(^\text{31}\) The deficiency of micronutrients and the innate antioxidant principles brings on the suppression of Immune function of the individual reflected as the defective antibody responses and the innate T-cell mediated responses resulting in Immuno compromised status and further opportunistic infections.\(^\text{32}\) Viral pathogenesis can lead to oxidative stress through alterations of biological structures, impaired of immune function, increased of viral replication and activation of inflammatory response.\(^\text{33,34}\) It may result in the production of inflammatory cytokines and increase of disease severity. During viral infection, a rapid production of IFNs is needed to prevent the spread of viruses in the host. The herbal drug DC was subjected to in vitro free radical scavenging assay (DPPH (2, 2- diphenyl 1-2-picyrylhydrazyl
method) and the IC50 Values of DC was 73.24 μg/ml ± 18.47 was compared with standard ascorbic acid (13.88 ± 0.93) considering the herbal drug DC as an antioxidant with intermediate potency. 35 Hence it can aid in the reduction of disease severity by managing the oxidative stress caused by the viral pathogens. Literature analysis of the ingredients of drug DC shows that it has anti retroviral as well as immunomodulatory activity thereby it can boost the host immune system. Molecular docking studies of DC when compared to that of the standard antiretroviral drug Nevirapine revealed reverse transcriptase enzyme inhibition ensuring its effect on reducing the viral replication. Inhibitors of reverse transcriptase activity remain a major target for viral research in drug discovery. 36 Previous research work in search for potent SARS-CoV protease inhibitors by VS Lee et al., performed the molecular docking studies and the preliminary results show that among sixteen antiviral drugs taken from the NCI database, four of them with trade name Nevirapine, Glycovir, Virazole, and Calanolide A, are observed to fit well in the active site of the SARS-CoV protease. 37 Considering the safety of DC, the toxicity evaluation of DC was also performed on acute and chronic toxicity in animal models. The treated animals survived throughout the study period and did not reveal any observable signs of toxicity. On necropsy, no abnormalities were observed. For the chronic toxicity, the treated animals did not shown any abnormal findings at both test dose levels. The values of haematological and biochemical parameters were within the normal limits, indicating that the drug exerted nil impact on the parameters. The necropsy studies showed no remarkable changes. In histo pathological studies, both mid dose and high dose treated rats shown no significant abnormalities. 38 Based on toxicity studies, the trial drug DC was found to be nontoxic when studied on animal Models.

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Fig 1 Proposed action of DC in Coronavirus replication process

CONCLUSION
Understanding general aspects of molecular biology and biochemistry, gaining a complete picture of the intricacies of understanding the unique RNA replication process and host immunopathological response used by these viruses will significantly improve our ability to reduce disease burden. Through this review, a preliminary effort has been laid to scientifically explore the antiretroviral, immunomodulatory action and safety of DC substantiating its prospective benefits in the prevention and management of Corona virus. Therefore it can be suggested to be used in combination with conventional treatment methods to prevent the morbidity and mortality of 2019-CoV. An integrated clinical study may be warranted in large scale to further confirm its therapeutic efficacy. This preliminary effort has highlighted safety and benefits of Siddha herbal antiviral formulation to the scientific community in this decisive antiviral search scenario of 2019-CoV.

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