Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations (not to exceed 6000 words).

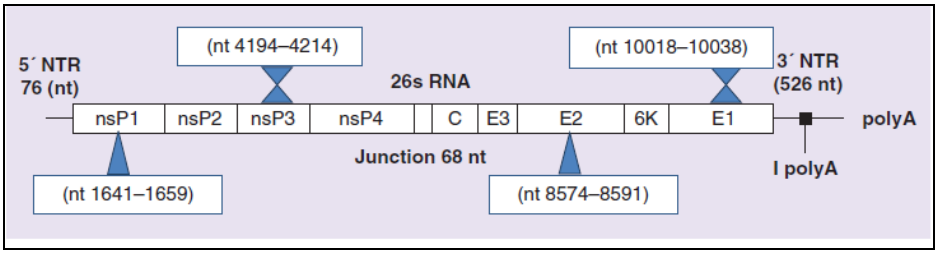
Dengue and chikungunya virus infections are important causes of morbidity and mortality in tropical and subtropical parts of the world. Dengue virus (DENV) and chikungunya virus (CHIKV) are transmitted through *Aedes aegypti* and *Aedes albopictus* mosquitoes. Both of the viruses cause acute febrile illness, and symptoms wise, both diseases are identical in the acute phase, though the clinical presentation differs as the infection progresses. There are no licensed antivirals/vaccines available against DENV and CHIKV, and their prevention is still based on vector control measures. Therefore, the need for effective drugs with anti-dengue and anti-chikungunya activities is imperative ***(Parashar et al 2014)***.
In the area of antiviral therapeutics, I have worked on RNA interference, natural & synthetic compounds, traditional medicinal plant extracts and repurposed drugs. The anti-dengue and anti CHIKV activity studies using *in vitro*/ *in vivo* assays is described below:

(A) RNA interference

RNA interference (RNAi) is the process of sequence-specific, post-transcriptional gene silencing (PTGS) in animals and plants, which is induced by 21- to 23-nucleotide (nt) small interfering RNA (siRNA) that is homologous in sequence to the silenced gene. RNAi not only regulates gene expression in the mammalian cells but also acts as a cellular defense mechanism against the invaders, including the viruses. Inhibition of specific genes by siRNAs has proven to be a potential therapeutic strategy against viral infection. For instance, inhibition of virus replication and gene expression by directly introducing siRNAs into the cells have been reported for several viruses ***((Parashar et al 2016, Panda et al 2021)***.

(i) RNAi agent for inhibition of Chikungunya virus:

We evaluated the efficacy of the siRNAs against ns1 and E2 genes of CHIKV both *in vitro* and *in vivo*. Four siRNAs each, targeting the E2 and ns1 genes were designed and evaluated for efficiency in inhibiting CHIKV growth *in vitro* and *in vivo*.

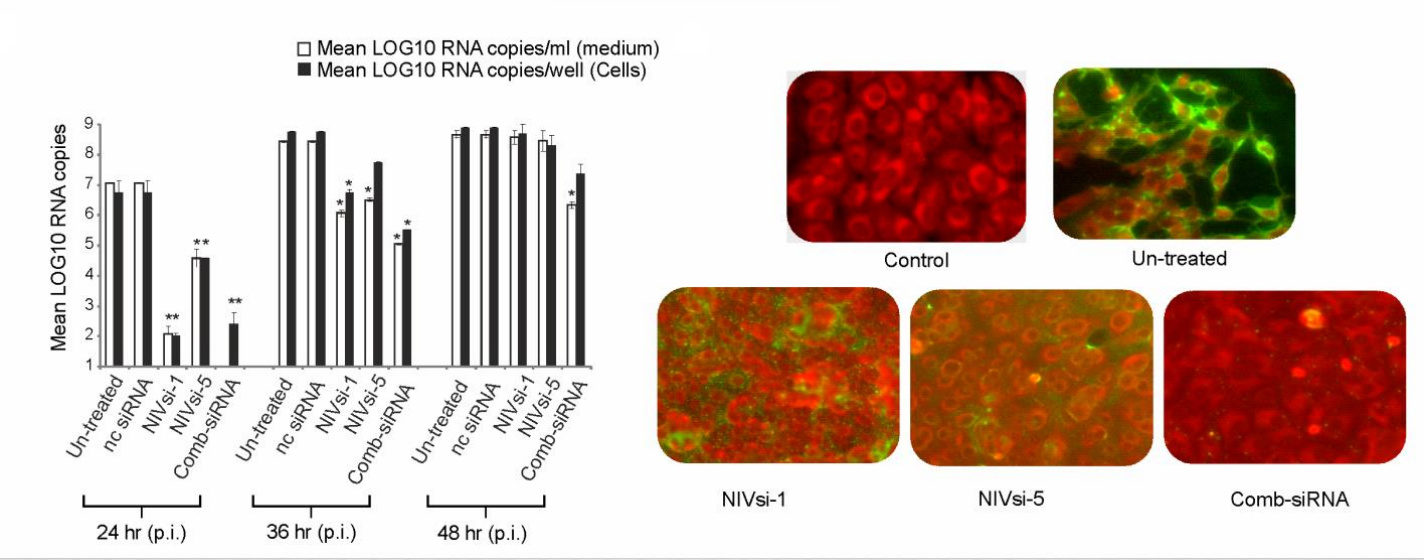


Sites of the siRNA target sequences within the chikungunya virus genome

Two siRNAs were effective in controlling CHIKV replication in vitro as assessed by different assays. CHIKV replication was completely inhibited in the virus-infected mice when administered 72 hours post infection (h.p.i.). The combination of two siRNAs exhibited additive effect leading to early and complete inhibition of virus replication. These findings suggest that RNAi capable of inhibiting CHIKV growth might constitute a new therapeutic strategy for

controlling CHIKV infection and transmission ***(Parashar et al 2013, Patent granted: US (2017), Europe (2019), China (2019), Australia (2021) & India (2021).***

In vitro evaluation: For the initial comparison of antiviral activity of different siRNAs, Vero-E6 cells were infected with CHIKV and transfected with different siRNAs (Chik-1 to Chik-8) 2 h p.i. Chik-1 and Chik-5 were the most effective siRNAs, suppressing CHIKV copies by 5 log10 and 2.5log10 RNA copies respectively. The pool of siRNAs Chik 1–4 (4 log10) and Chik5–8 (3 log10) did not increased the CHIKV suppression in Vero E6 cells. Results obtained with the individual siRNAs and pool of siRNAs indicated that only siRNAs Chik-1 and Chik-5 possessed the antiviral activity against CHIKV. Therefore, only Chik-1 and Chik-5 and Comb-siRNA were used for further studies. The reduction in the CHIKV copies by Chik-1 and Chik-5 was initiated at the siRNA concentrations of 50 pmol, and reached a plateau at 100 pmol. Chik-1 and Chik-5 showed sequence dependent inhibition and showed no reduction in the dengue-2 and the Chandipura virus replication in Vero-E6 cells.



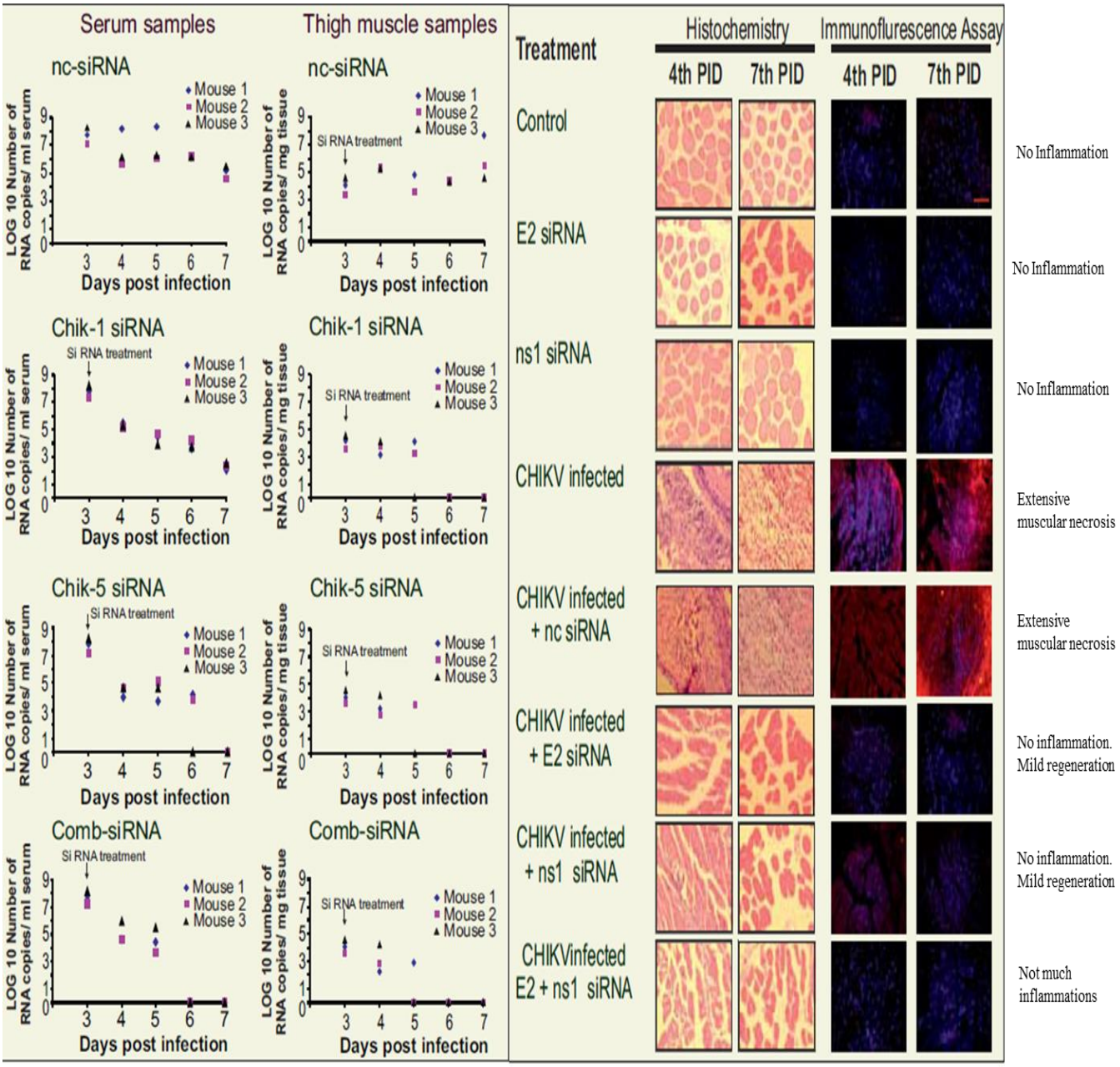
Quantitative analysis of intra cellular and extra cellular CHIKV RNA copies using real time PCR

Immuno-fluorescence assay

Evaluation of siRNAs directed against CHIKV

In vivo evaluation: Chik-1, Chik-5, Comb-siRNA and ncsiRNA administered 72 h p.i. provided significant reduction in the serum viral load as assessed daily by real time PCR. At 24 h and 48 h post siRNA treatment, 2.5 log10 and 3.5 log10 reduction was recorded for all siRNAs, when compared to ncsiRNA. At 72 h post treatment, reduction with siRNA Chik-1, and Chik-5 was around 3.5 log10 while CombsiRNA showed 100% inhibition (7log10). Importantly, Comb-siRNA produced prolonged inhibitory effect when compared to individual siRNAs. In muscle tissues, CHIKV RNA reached peak by third day p.i., with viral loads ranging from 16104 to 76105 viral RNA copies/mg tissue. At 24 h post-siRNA treatment ,2.5 log10 reduction in CHIKV RNA was noted with all the three siRNAs as compared to ncsiRNA control. At 72 h, all the siRNAs produced 4log10 reduction in CHIKV RNA (100% inhibition). Similar results were

seen when IFA was used to evaluate the effect of siRNA on CHIKV replication in muscle tissues that corroborated with real time PCR-based data ***(Parashar et al 2013)***.

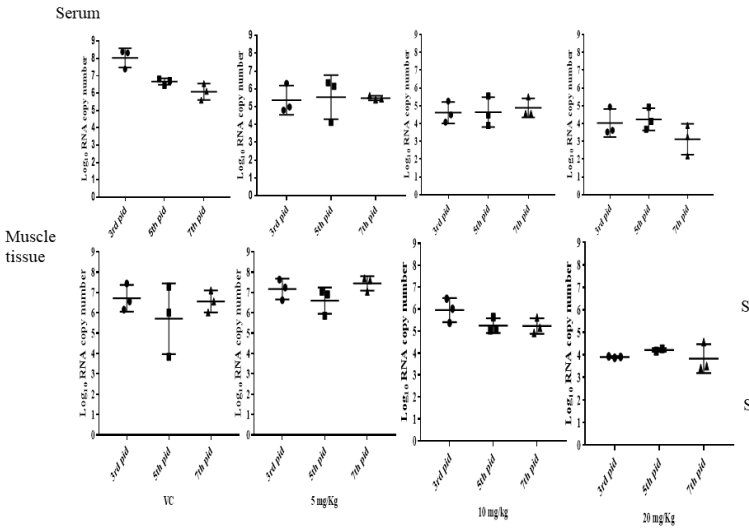


The reduction in CHIKV copies/ml serum after injection with siRNA Chik-1 and Chik-5 in C57BL/6 mice infected with CHIKV

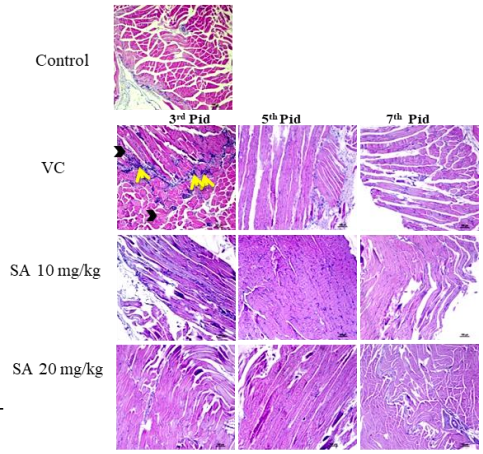
(ii) Development of delivery systems for targeted delivery of siRNAs for chikungunya treatment

The real therapeutic potential of siRNA is limited due to the fact that unprotected oligonucleotides such as - unmodified siRNA for ns1 and E2 genes have a very short half-life *in vivo* (seconds to minutes) due to degradation (by endogenous nucleases) and rapid kidney filtration from circulation (due to their small size). For these reasons, enhanced stability and site specific siRNA delivery strategies through employment of novel nanoparticle based delivery vehicles are promising because nanoparticles prevent degradation or rapid clearance, enhance uptake and promote endosomal escape of therapeutic siRNA ***(Parashar et al 2020)***. However, it is important that the nanoparticle vehicles for drug delivery must demonstrate siRNA binding, low cytotoxicity, effective cellular uptake, and, most importantly, evidence of siRNA-induced knockdown. Hence we attempt to develop therapeutic siRNA encapsulating solid lipid nanoparticles (SLN) & Zeolitic imidazolate framework for CHIKV antiviral therapy.

Cationic lipids: For the first time we have reported the efficacy of Stearylamine (SA), a cationic lipid alone against CHIKV. Our findings demonstrate the potential of SA in effectively inhibiting the viral replication in infected Vero cells thereby decreasing the viral load in C57BL/6 mice. SA has a direct effect and helps ameliorate acute disease symptoms in CHIKV-infected mice. Promising results from this study frames a commensurate basis to develop a suitable delivery system along with a drug or gene against chikungunya as a novel antiviral therapy. This preclinical study provides evidence to support further studies to develop SA based formulations to treat chikungunya fever effectively ***(Jeengar et al 2021)***.



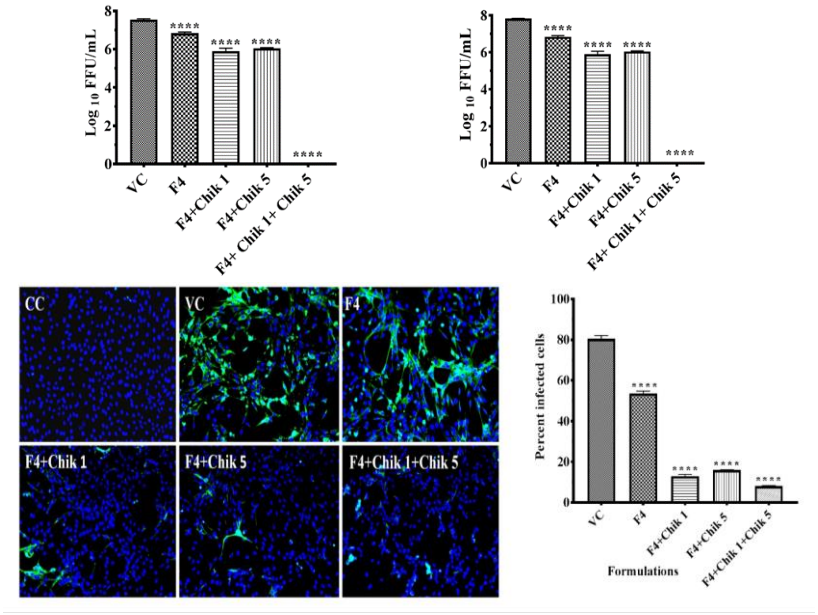
The reduction in CHIKV copies/mL in serum and muscle tissue

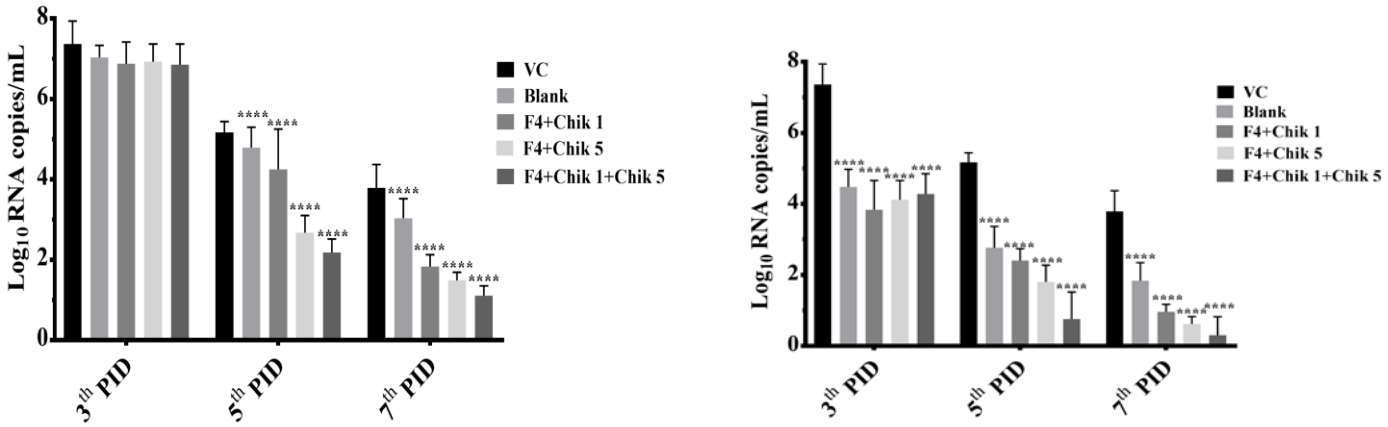


Histopathological changes in mouse muscle tissues after chikungunya infection and SA treatment

Soli Lipid Nanoparticles:

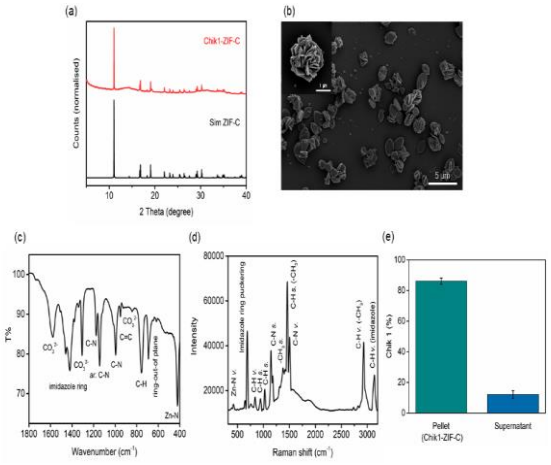
Nanodelivery systems were prepared, characterized and complexed with siRNA targeting E2 and NS1 gene region. The developed four delivery systems (F1,F2,F3 and F4) were assessed for stability and potential toxicities against CHIKV. In comparison to the other nanodelivery systems, F4 having allowing maximum siRNA complexation, better stability and higher transfection with strong inhibition against E2 and NS1 genes of CHIKV. The study concludes that cationic lipid like stearylamine with ease of synthesis and characterization, indicated maximum complexation by structural condensation of siRNA owing high transfection alone and synergistic inhibition of CHIKV along with siRNA both *in-vitro* and *in-vivo* models. Therefore, stearylamine based cationic lipid nanoparticles can be embraced and explored as safe, potent and efficient no viral vectors overcoming siRNA *in-vivo* complexities against chikungunya. In future, nanoparticles containing siRNA approach can be used in developing delivery system for the treatment of other viral disease treatment ***(Jeengar et al 2022)***.



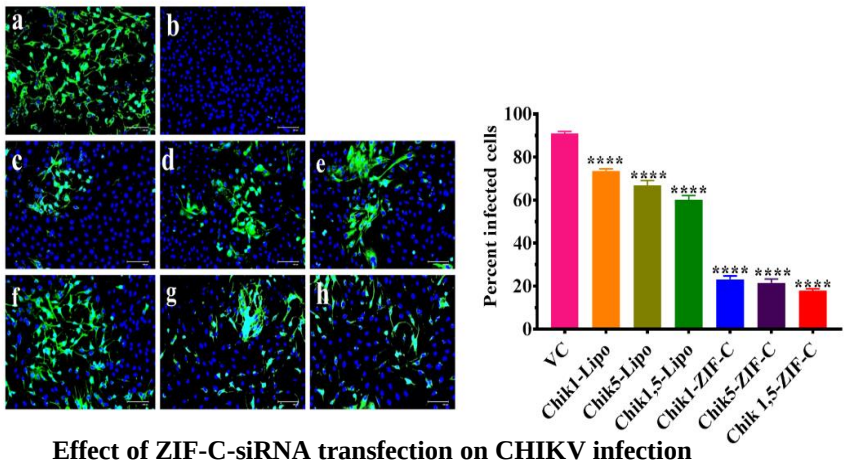


Zeolitic imidazolate framework:

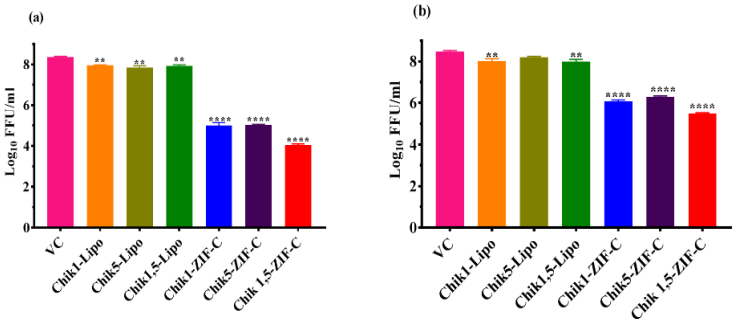
The therapeutic efficiency of siRNA can be improved by using an efficient delivery system. Metal-organic framework biocomposits have demonstrated an exceptional capability in protecting and efficiently delivering nucleic acids into cells. In the present study, carbonated ZIF called ZIF-C has been utilized to deliver siRNAs targeted against E2 and nsP1 genes of CHIKV to achieve a reduction in viral replication and infectivity. Cellular transfection studies of E2 and nsP1 genes targeting free siRNAs and ZIF-C encapsulated siRNAs in CHIKV infected Vero CCL-81 cells were performed. Our results reveal a significant reduction of infectious virus titre, viral RNA levels and percent of infected cells in cultures transfected with ZIF-C encapsulated siRNA compared to cells transfected with free siRNA. The results suggest that delivery of siRNA through ZIF-C enhances the antiviral activity of CHIKV E2 and nsP1 genes directed siRNAs ***(Tagore et al 2022).***

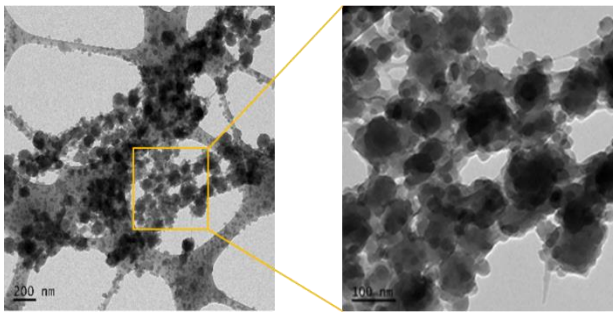


Characterization of Chik1-ZIF-C

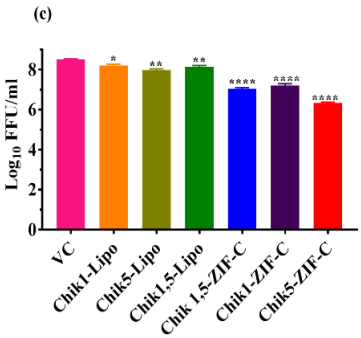


Effect of ZIF-C-siRNA transfection on CHIKV infection





TEM image of Chik1-ZIF-C



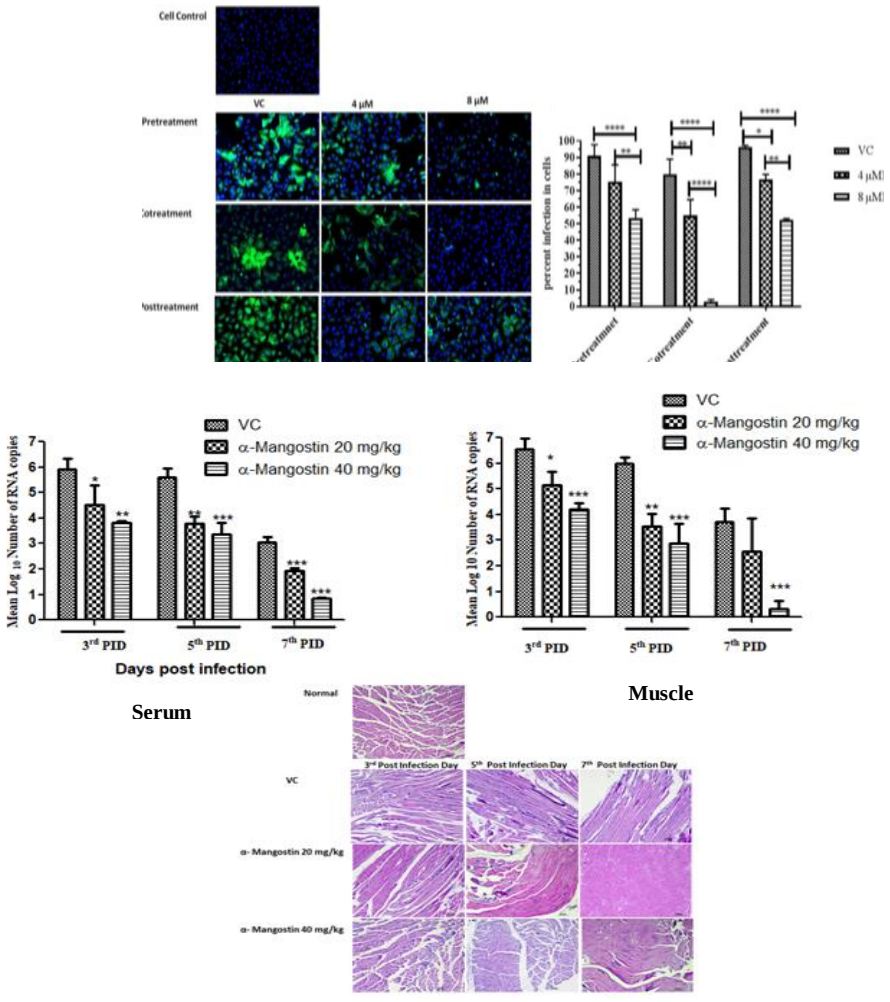
Time of addition dependent inhibitory effects

(B) ***Natural/synthetic compounds:***

I) Effective against chikungunya & dengue virus

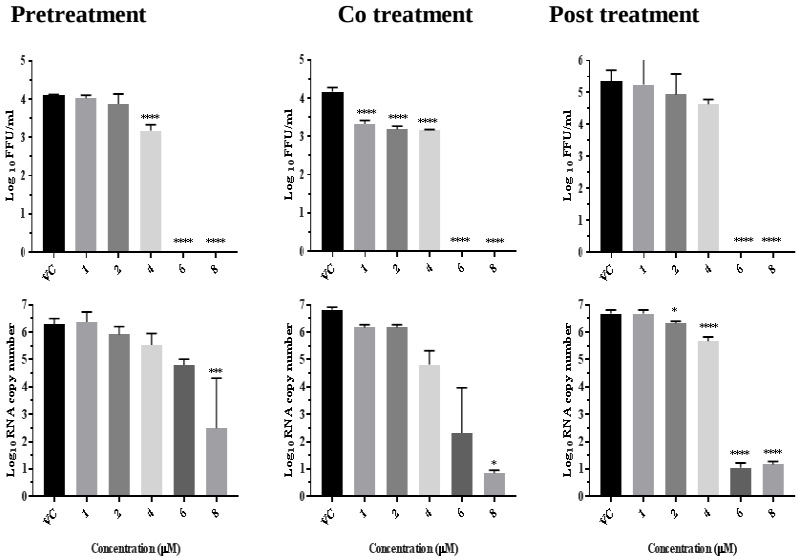
i) ***α-Mangostin:*** As diverse natural phenolic compounds have been shown to possess antiviral activities, we explored the antiviral activity of compounds, shortlisted using an *in-silico* near-neighbor search within the National MolBank repository of IICT, Hyderabad, against CHIKV both *in vitro* and *in vivo*.

In vitro studies revealed that α-Mangostin completely inhibited CHIKV infectivity under the cotreatment condition. CHIKV replication was also inhibited in virus-infected mice. This is the first *in vivo* study which clearly showed that α-Mangostin is effective *in vivo* by significantly reducing virus replication in serum and muscles. Molecular docking indicated that α-Mangostin can efficiently interact with the E2–E1 heterodimeric glycoprotein and the ADP-ribose binding cavity of the nsP3 macrodomain. The findings suggest that α-Mangostin can inhibit CHIKV infection and replication through possible interaction with multiple CHIKV target proteins and might act as a prophylactic/therapeutic agent against CHIKV ***(Patil et al 2021)***.

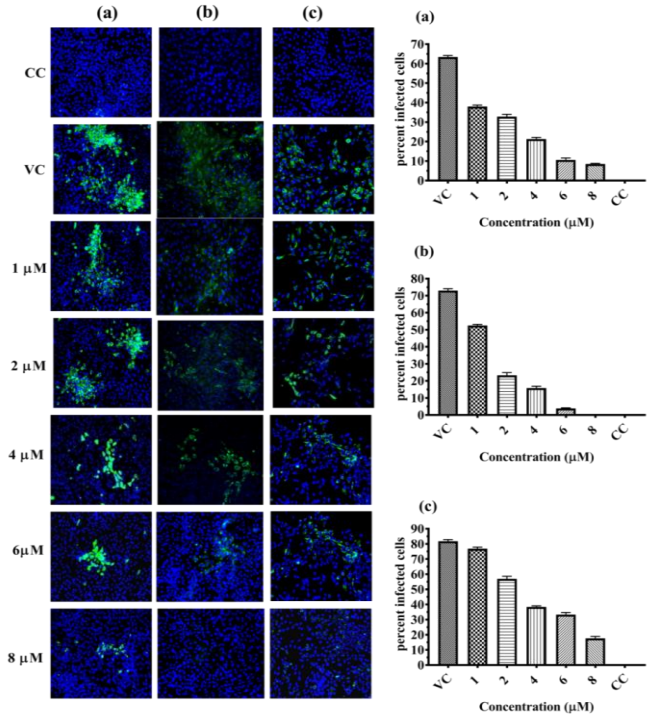


Histopathological changes in mouse muscle tissues

Natural compounds were also screened for their antiviral activity against DENV by *in vitro* cell line-based assay. α-Mangostin, a xanthanoid, was observed to exert antiviral activity against DENV-2 under pre-, co- and post-treatment testing conditions. The in vitro and in silico findings suggest that α-Mangostin possesses the ability to suppress DENV-2 production at different stages of its replication cycle and might act as a prophylactic/therapeutic agent against DENV-2 ***(Panda et al 2021)***.



Focus forming unit assay



Immuno-fluorescence assay

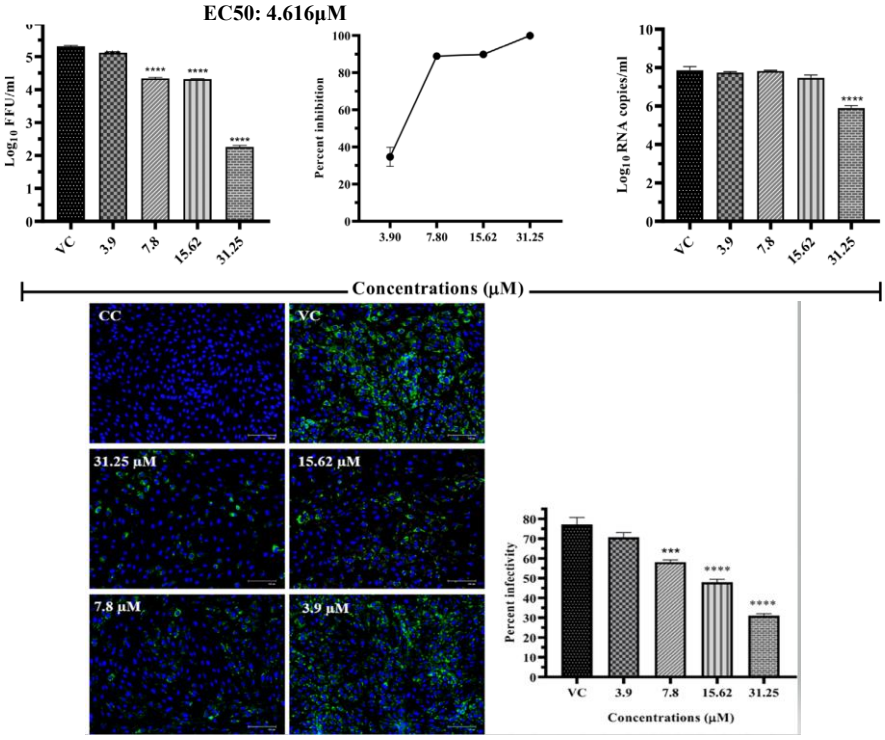
<u>Effect of α-Mangostin on DENV-2</u>

II) Effective against dengue virus

ii) *Carpaine*:

Traditional remedies are being used to treat dengue fever and many studies have reported the utilization of *Carica papaya* leaves extracts (CPLE) in treating dengue patients. CPLE have been extensively used to treat thrombocytopenia in several cases. Among the compounds reported from CPLE, carpaine is a major alkaloid and active compound. Carpaine has been reported to have antithrombocytopenic activity.

Antiviral activity was observed under post-treatment conditions. The highest reduction was observed in infected cultures treated with 31.25µM of carpaine (from 5.318 in VC to 2.259 mean log10 FFU/ml). In infected cell cultures treated with 15.62 and 7.8 µM carpaine concentrations, one log reduction in virus titre was observed. Carpaine treatment led to a significant 2 log10 titre decrease in viral RNA copy number at 31.25 µM concentration compared to VC. In IFA, carpaine significantly decreased the percent of infected cells compared to VC. The findings suggest the anti-DENV property of carpaine post infection. The anti-DENV activity was confirmed by different assays which measure infectious virus titre, viral RNA copy number, and viral protein expression. The *in-silico* observation of stable binding of carpaine with NS5 RdRp suggests that carpaine might interfere with the functioning of viral replication. To conclude, the present study provides *in-vitro* and *in-silico* evidence of anti-DENV activity of carpaine against DENV-2 in Vero CCL-81 cells ***(Alagarasu et al 2023)***.

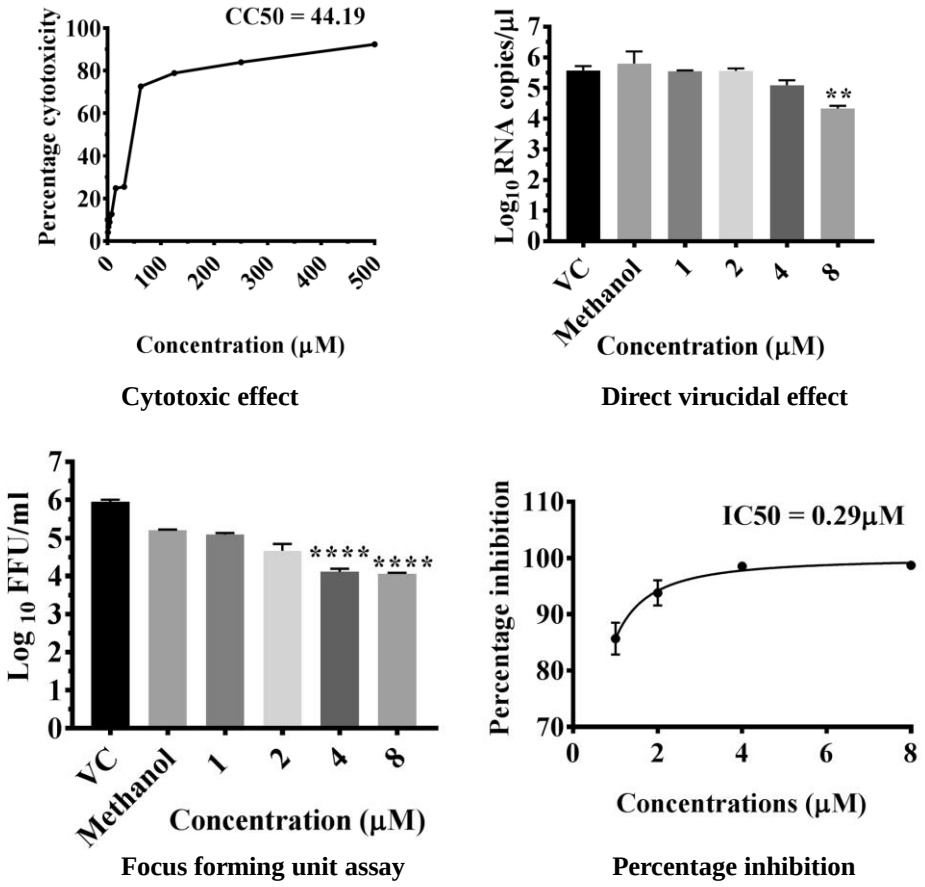


Immunofluorescence assay

Effect of carpaine on DENV-2 titre and RNA levels under post-treatment condition

III) Effective against chikungunya virus

iii) *Chebulinic acid:* This is originally isolated from the flower extract of the plant *Terminalia chebula,* has been shown to inhibit infection of herpes simplex virus-2 (HSV-2), suggestively by inhibiting the host entry step of viral infection. Like HSV-2, the DENV and CHIKV also use receptor glycosaminoglycans (GAG) to gain host entry, therefore, the activity of Chebulinic acid (CA) was tested against these viruses. Co-treatment of 8 µM CA with DENV caused 2 log decrease in the virus titer (4.0 log10FFU/mL) at 120 h post infection, compared to virus control (5.95 log10FFU/mL). In contrast, no inhibitory effect of CA was observed against CHIKV infection under any condition. The mechanism of action of CA was investigated in silico by employing DENV and CHIKV envelope glycoproteins. During docking, CA demonstrated equivalent binding at multiple sites on DENV envelope protein, including GAG binding site, which have previously been reported to play a crucial role in host attachment and fusion, indicating blocking of these sites. However, CA did not show binding to the GAG binding site on envelope protein-2 of CHIKV. The *in vitro* and *in silico* findings suggest that CA possesses the ability to inhibit DENV infection at the entry stage of its infection cycle and may be developed as a potential therapeutic agent against it ***(Thomas et al 2022)***.



Effect of Chebulinic acid on Vero E6 cells and dengue virus

(C)Traditional medicinal plant extracts

Though a number of plants have been tested for the antiviral activity, it would be worth investigating other plants that have been used in the age-old practices in Ayurveda, which is a traditional form of Indian medicine. There is a need to study the antiviral activity of those herbs which are used in traditional forms of medicine for which not much scientific evaluations have been carried out. Furthermore, it would be important to evaluate the Indian alternatives to the plants that are not native to India having anti-dengue and anti-chikungunya activities. Therefore, extracts using different plants with medicinal properties were tested for their antiviral activity against dengue and chikungunya virus.

i) *Plumeria alba:* The chloroform extract from Plumeria alba bark showed 100% activity against DENV under posttreatment conditions and methanol extract from *Plumeria alba* leaves showed 100% activity against CHIKV. The results suggest the therapeutic utility of *Plumeria alba* against both of these viruses ***(Alagarasu et al 2022)***.

ii) *Bacopa monnieri:* Whole herb of *Bacopa monnieri* showed a significant reduction of DENV and CHIKV titers under posttreatment conditions ***(Alagarasu et al 2022)***.

iii) *Ancitrocladus heyneanus:* Extract from *Ancitrocladus heyneanus* bark showed total reduction of viral foci of DENV and significant activity against CHIKV ***(Alagarasu et al 2022)***.

iv) Vitex negundo: Leaves of *Vitex negundo* showed a significant reduction of DENV, while no significant activity was found against CHIKV ***(Alagarasu et al 2022)***.

v) *Curcubita maxima*: Seeds of *Curcubita maxima* showed a 100% reduction in the case of CHIKV ***(Alagarasu et al 2022).***

vi) *Carica papaya:* The current study was undertaken to study the antiviral activity of commercially available *Carica papaya* leaves extract (CPLE) based products and CPLE prepared in four formulations against DENV and CHIKV. Maximum nontoxic concentrations of the commercially available CPLE based products and CPLE based formulations (*Carica papaya* leaves in powder form, *Carica papaya* leaves in lyophilized form, *Carica papaya* leaves based silver nanoparticles and supercritical fluid extract of *Carica papaya* leaves) were used for screening the antiviral activity. The antiviral activity against DENV-2 and CHIKV were assessed post infection using focus forming unit assay. Effective formulations were tested under different conditions i.e. pretreatment, cotreatment and posttreatment. The virus output after treatment was assessed by real-time RT-PCR, immunofluorescence assay and focus forming unit assay. The results revealed *Carica papaya* leaves based silver nanoparticles and supercritical fluid extract of *Carica papaya* leaves formulations showed significant inhibition in case of DENV while papaya leaves in powder form showed significant reduction in case of CHIKV. This study demonstrates the antiviral activity of CPLE formulations against DENV and CHIKV infection in in-vitro system and needs further validation in *in-vivo* models ***(Patil et al 2022, Shrivastava et al 2022)***.

vii) *Sauropus androgynus:* commonly known as "multigreen" and "multivitamin" is consumed as a vegetable and used in traditional medicine to relieve fever. This *in vitro* study is aimed to explore the activities of the lipophilic fraction of the leaves of S. androgynus (LFSA) against DENV, CHIKV and malaria (*P. falciparum* strain 3D7) parasite. The LFSA was analyzed by using GC-FID and GC-MS. The antiviral activity of LFSA was studied using the Vero CCL-81 cell line. The cytotoxicity assay was performed using 3-(4,5-dimethythiazol-2-yl)- 2,5-diphenyl tetrazolium bromide. Focus forming unit, cell-based immunofluorescence assays, and quantitative RT-PCR, were used to determine and confirm antiviral activity against DENV and

CHIKV. The antiparasitic activity of LFSA was carried out against *P. falciparum* strain 3D7 grown in fresh O+ human erythrocytes culture. Twelve compounds were identified in LFSA using GC/MS. The most abundant compound was squalene (36.9%), followed by vitamin E (12.5%) and linolenic acid (10.2%). Significant reduction in DENV titre was observed under pre- and post-infection treatment conditions at a concentration of 31.25 µg/ml, but no antimalarial and anti-CHIKV activity was observed. The Autodock-Vina-based in-silico docking study revealed that β-sitosterol could form a strong interaction with the DENV E glycoprotein. Our findings suggest that LFSA can inhibit DENV infection and might act as a potent prophylactic/ therapeutic agent against DENV. In-silico results suggested that β-sitosterol may block the viral entry by inhibiting the fusion process ***(Joshi et al 2023)***.

viii) <u>Ocimum basilicum</u>: This plant is used to cure many types of fever in traditional medicine. This study aims to explore the antiviral activity of the lipophilic fraction of the stem of O. basilicum (LFOB) against DENV and CHIKV. The LFOB was analyzed using GC-FID and GC-MS. The antiviral activity of LFOB was studied using the Vero CCL-81 cell line. The cytotoxicity assay was performed using 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide. *In vitro* antiviral activity and FFU assay were used to determine and confirm antiviral activity against DENV and CHIKV. Twenty-six compounds were identified in LFOB using GC/MS. The most abundant compounds were β-sitosterol (22.9%), stigmasterol (18.7%), and campesterol (12.9%). Significant reduction in DENV titre was observed under pre- and post-infection treatment conditions at a concentration of 3.125 µg/mL, but no anti-CHIKV activity was observed. Our earlier and the present AutoDock-Vina-based in silico docking study revealed that β-sitosterol and stigmasterol could form strong interactions with the DENV E glycoprotein and DENV RdRp domain, respectively. Our findings suggest that LFOB can inhibit DENV infection and might act as a potent prophylactic/therapeutic agent against DENV-2. In silico results suggested that β-sitosterol and stigmasterol may block the viral entry by inhibiting the fusion process and viral replication respectively ***(Joshi et al 2023)***.

Medicinal plant extracts showing inhibition of DENV and CHIKV under different treatment conditions

Plant	Local name & plant part used	Maximum Concentration (µg/ml)	Log difference effectiveness against DENV	Log difference effectiveness against CHIKV
Plumeria alba	Champa (bark)	7.8	Cotreatment: 1.022 Posttreatment: 5.108	-
Plumeria alba	Champa (leaves)	250	Cotreatment: 2.096 Posttreatment: 1.285	Pretreatment: 7.87 Posttreatment: 7.564
Vitex negundo	Nirgundi (leaves)	7.8	Cotreatment: 2.432 Posttreatment: 2.224	-
Ancistrocladus heyneanus	Kardal (leaves)	3.9	Posttreatment: 5.108	Pretreatment: 2.536 Posttreatment: 3.327
Bacopa monnieri	Brahmi (whole herb)	125	Cotreatment: 2.187 Posttreatment: 5.108	Posttreatment: 1.311
Cucurbita maxima	Pumpkin (seeds)	250	-	Pretreatment: 7.878 Cotreatment: 7.569 Posttreatment: 7.564
Carica Papaya	Papaya extract based nanoparticles	100	Pretreatment: 2.43 Cotreatment 2.99 Posttreatment:5.72	-
	Supercritical fluid extract of papaya	100	Pretreatment: <1 Co-treatment: <1	-

			Post treatment: 1	
	Papaya leaves in powder	100	-	Post treatment: 3.65 Pretreatment: 1.38
Sauropus androgynus	Katuk (leaves)	31.25	Pretreatment: 1.308 Posttreatment: 1.447	-
Ocimum basilicum	Tulsi (Stem)	31.25	Posttreatment: 1.213	Co-treatment: 1

Repurposed drugs

Among the many approaches employed to identify drugs to treat dengue and chikungunya, drug repurposing has gained popularity, which is safer and more economic. The targeted drugs have further been tested for their effectiveness against other diseases and proven safe for treatment of the human diseases. In recent years, drug repurposing has been applied in many studies to identify treatments.

We employed a transcriptomics-based bioinformatics approach for drug identification against DENV. Gene expression omnibus datasets from patients with different grades of dengue disease severity and healthy controls were used to identify differentially expressed genes in dengue cases, which were then applied to the query tool of Connectivity Map to identify the inverse gene–disease–drug relationship. A total of sixteen identified drugs were investigated for their prophylactic, virucidal, and therapeutic effects against DENV. Focus-forming unit assay and quantitative RT-PCR were used to evaluate the antiviral activity. Results revealed that five compounds, viz., resveratrol, doxorubicin, lomibuvir, elvitegravir, and enalaprilat, have significant anti-DENV activity. Further, molecular docking studies showed that these drugs can interact with a variety of protein targets of DENV, including the glycoprotein, the NS5 RdRp, NS2B-NS3 protease, and NS5 methyltransferase The in vitro and in silico results, therefore, reveal that these drugs have the ability to decrease DENV-2 production, suggesting that these drugs or their derivatives could be attempted as therapeutic agents against DENV infections ***(Punekar et al., 2022).***

Summary of effective inhibition under different treatment conditions

Sr. no.	Drug name	CC50 (µM)	Maximum concentration (µM)	Log difference effectiveness against DENV-2	EC50 (µM)	Selectivity Index (SI)
1	Doxorubicin	25	25	Post-treatment- 1.453 Co-treatment- 4.958	19.99 6.573	5.848 17.785
		12.5	12.5	Co-treatment- 4.377	6.573	17.785
		6.25	6.25	Co-treatment- 2.107	6.573	17.785
2	Resveratrol	12.5	12.5	Post-treatment- 1.226 Pre-treatment- 1.188	4.013 7.592	10.102 5.340
		6.25	6.25	Post-treatment- 1.115	4.013	10.102
3	Enalaprilat	1.56	1.56	Post-treatment- 1.239	1.079	74.911
4	Elvitegravir	6.25	6.25	Pre-treatment- 1.316	4.405	2.883
5	Lomibuvir	6.25	6.25	Pre-treatment- 1.224	3.740	10.305

Anti CHIKV activity of fourteen FDA-approved drugs was investigated by *in vitro* and *in silico* approaches. The findings showed that nine compounds, viz., temsirolimus, 2-fluoroadenine, doxorubicin, felbinac, emetine, lomibuvir, enalaprilat, metyrapone and resveratrol exhibit anti CHIKV activity. Furthermore, *in silico* molecular docking studies revealed that these drugs can bind to structural protein targets such as envelope protein, and capsid, and nonstructural proteins NSP2, NSP3 and NSP4 (RdRp). Findings from *in vitro* and *in silico* studies reveal that these drugs can suppress the infection and replication of CHIKV and further *in vivo* studies

Among the drugs which showed anti CHIKV activity in the present study, resveratrol, doxorubicin, lomibuvir and enalaprilat were also reported to exert anti-DENV activity (Punekar et al., 2022). These drugs might be useful in regions where both viruses are endemic and need to be prioritized. Apart from the drugs with anti-DENV activity, temsirolimus can be taken forward since, the inhibitory effect against CHIKV was greater compared to other drugs. The results of the current investigation could serve as the foundation for *in vivo* studies that examine the possibility of treating chikungunya fever with FDA-approved drugs by drug repurposing ***(Kasbe et al 2023)***.

Sr. no.	Drug name	CC50 (µM)	Maximum concentration (µM)	Log difference effectiveness against CHIKV	EC50 (µM)	Selectivity Index (SI)
1	Temsirolimus	413.4	50	Pretreatment- 2.316 Posttreatment- 5.192	4.857 6.043	85.11 68.41
			25	Pretreatment-1.386 Posttreatment- 0.967	4.857 6.043	85.11 68.41
2	2-fluoroadenine	436.7	100	Pretreatment- 1.48 Posttreatment- 1.184	9.586 7.592	45.56 5.340
			50	Pretreatment- 1.381 Posttreatment- 1.187		
3	Doxorubicin	116.9	25	Pretreatment- 1.332 Posttreatment-1.494	6.896 6.408	16.95 18.24
			12.5	Pretreatment- 1.205 Posttreatment- 1.361		
			6.25	Posttreatment- 1.112		
4	Felbinac	525	3.125	Pretreatment- 1.625	0.238	2205.88
			1.56	Pretreatment- 1.49		
5	Metyrapone	5599	200	Pretreatment- 1.55	12.23	457.80
			100	Pretreatment- 1.529		
6	Enalaprilat	83.04	1.56	Cotreatment- 1.142	0.496	167.42
			0.78	Cotreatment- 0.897		
7	Emetine	>200	200	Posttreatment- 1.564	4.237	47.2
			100	Posttreatment-1.32	4.237	47.2
			50	Posttreatment-1.068	4.237	47.2
8	Resveratrol	40.86	12.5	Posttreatment- 1.384	1.721	23.74
			6.25	Posttreatment- 0.609	1.721	
9	Lomibuvir	31.48	6.25	Posttreatment- 1.215	1.587	19.83

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