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A Review: Zika Virus updates

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Abstract

Zika virus is a Single-stranted RNA Arbovirus of the Flaviviirus genus and Flaviviridae family which is primarily transmitted by Aedes genus mosquito. In Uganda, it was initially discovered in monkeys in 1947 and then in human in 1952. After a few days, outbreaks were observed in a number of nations, including those in Asia, Africa, America, and the Pacific. And in 2016 the WHO declared microcephaly and other brain diseases as a Public Health Emergency of International Concern (PHEIC). Mild Zika virus symptoms like fever, exhaustion, headaches, muscle soreness, and conjunctivitis are the most common, whereas severe infections can cause neurological abnormalities such Guillain-Barre syndrome and prenatal microcephaly. Although researchers put a lot of work into creating efficient defences against zika virus, there is still no specific antiviral medication that has been authorized for the treatment of zika virus infection. The primary method for preventing the breeding of mosquitoes is to take preventive measure against infection. Studies have specifically shown the potential of natural materials as ZIKV infection antivirals. As a result, this paper will examine current developments in the discovery of zika virus antiviral drugs using natural ingredients and also the immuneboosters as the preventive measure to strengthen the immune system and guard against zika virus infection.

Key words:

Zika virus; Virology; Cellular mechanism; Natural antivirals; Immuneboosters.

INTRODUCTION

Zika virus is mosquito borne, single stranded RNA virus belongs to Flavivirus genus of Family Flaviviridae. It was first discovered in 1947 in rhesus monkeys in forest of Uganda from South Africa. And later it first detected in human in 1954 in Nigeria. It mainly transmitted through two ways i.e. Vector borne transmission and non-vector borne transmission. Aedes genus mosquitos are main vector for these viruses, mostly the Aedes aegypti and other like Aedes albopictus and Aedes africanus. In tropical and subtropical regions.aedes genus mosquitoes normally bite during the day, peaking during early morning and late afternoon/evening. ZIKV is closely related to dengue virus (DENV), yellow fever virus (YFV), West Nile virus(WNV), Japanese encephalitis virus(JEV), Tick borne

encephalitis. Non-vector transmission including sexual transmission, blood transfusion, organ transplantation and also mother to child transmission during pregnancy or during delivery is a potential route of transmission. Vertical/interuterine transmission leads to congenital infection and causes microcephaly in which there is a incomplete brain development and congential ZIKV syndrome. It also causes autoimmune disorder like Guillain-Barre Syndrome in which the immune system damage the peripheral nervous system.

Virology

ZIKV is a positive-single stranded RNA virus comprised of an envelope and a nucleocapsid. Virus particles are small, spherical and have icosahedral symmetry. ZIKV genome size is approximately 10.8kb which is translated into single polyprotein (3423 amino acids Length). The virus has two untranslated region i.e. 5' UTR and 3'UTR which consist of 100 nucleotide and 420 nucleotide respectively. The ZIKV virion consist of three structural proteins i.e. Capsid [C], envelope [E], premembrane [prM] and seven non-structural proteins i.e. NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5.

Structurally, E and M proteins are present at the surface of the viral particles, while the nucleocapsid is made up of C protein and the genomic RNA molecule. E is involved in different aspects of the viral cycle, mediating binding, membrane fusion. The NS5 protein is a largest viral protein whose C terminal portion has RNA dependent RNA polymerase (RdRp) activity and N terminal performs important role in RNA capping by virtue of its processing due to Methyl transferase activity and Guanylyl transferase activity (MTase and GTase). And NS3 protein consist serine protease and RNA helicase domains. NS2B is essential co-factor of NS3 protease and NS4B blocks the alpha and beta interferon signaling.

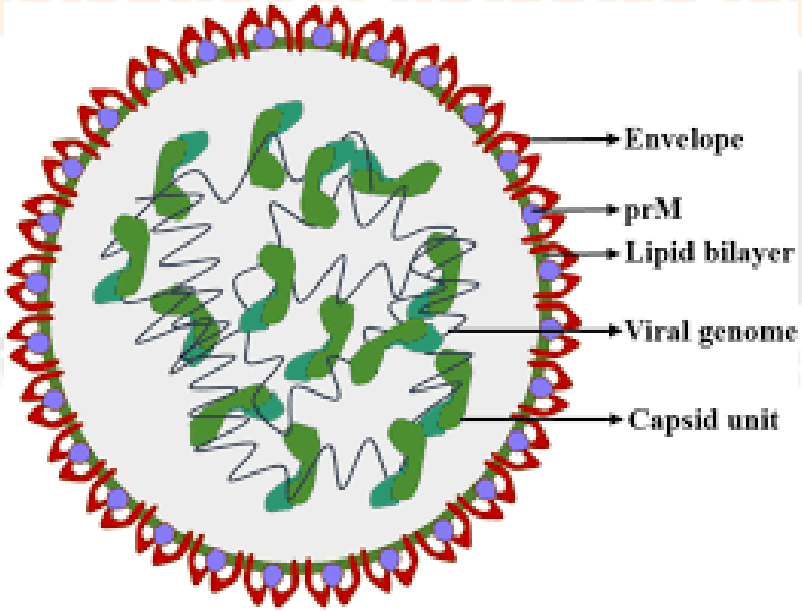


Fig. No.1.Zika virus structure

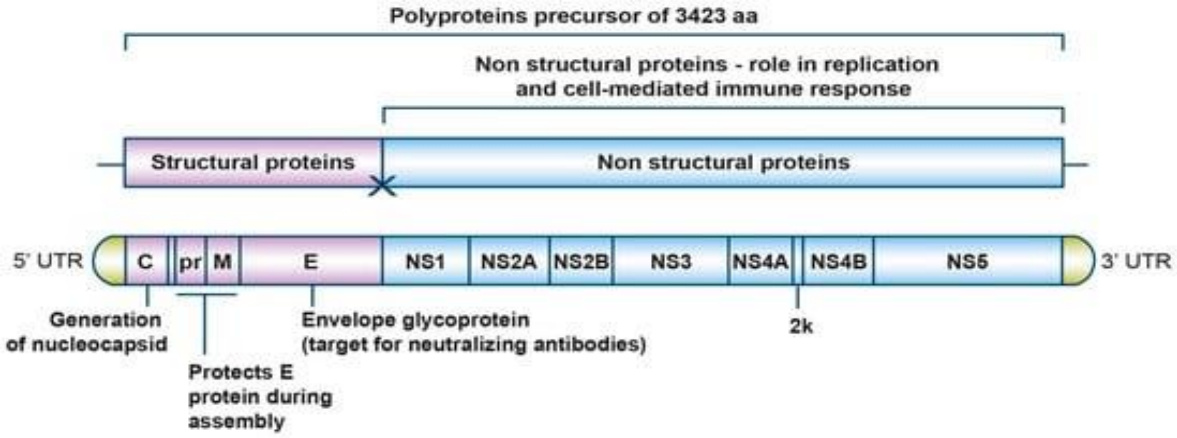


Fig. No.2. Genome structure of Zika virus

Pathogenesis

ZIKA virus replicates in the mosquito mid gut in epithelial cell and then in salivary gland. After replication 5 to 10 days the virus can be found in the saliva of mosquito. If the mosquito saliva is inoculated into human skin, the virus can infect epidermal keratinocytes, skin fibroblast and the Langerhans cells. After that the pathogenesis of the virus continues to spread to lymph nodes and the blood stream. ZIKA antigens have been found in cell nuclei.

Pathophysiology

- Based on investigational studies:

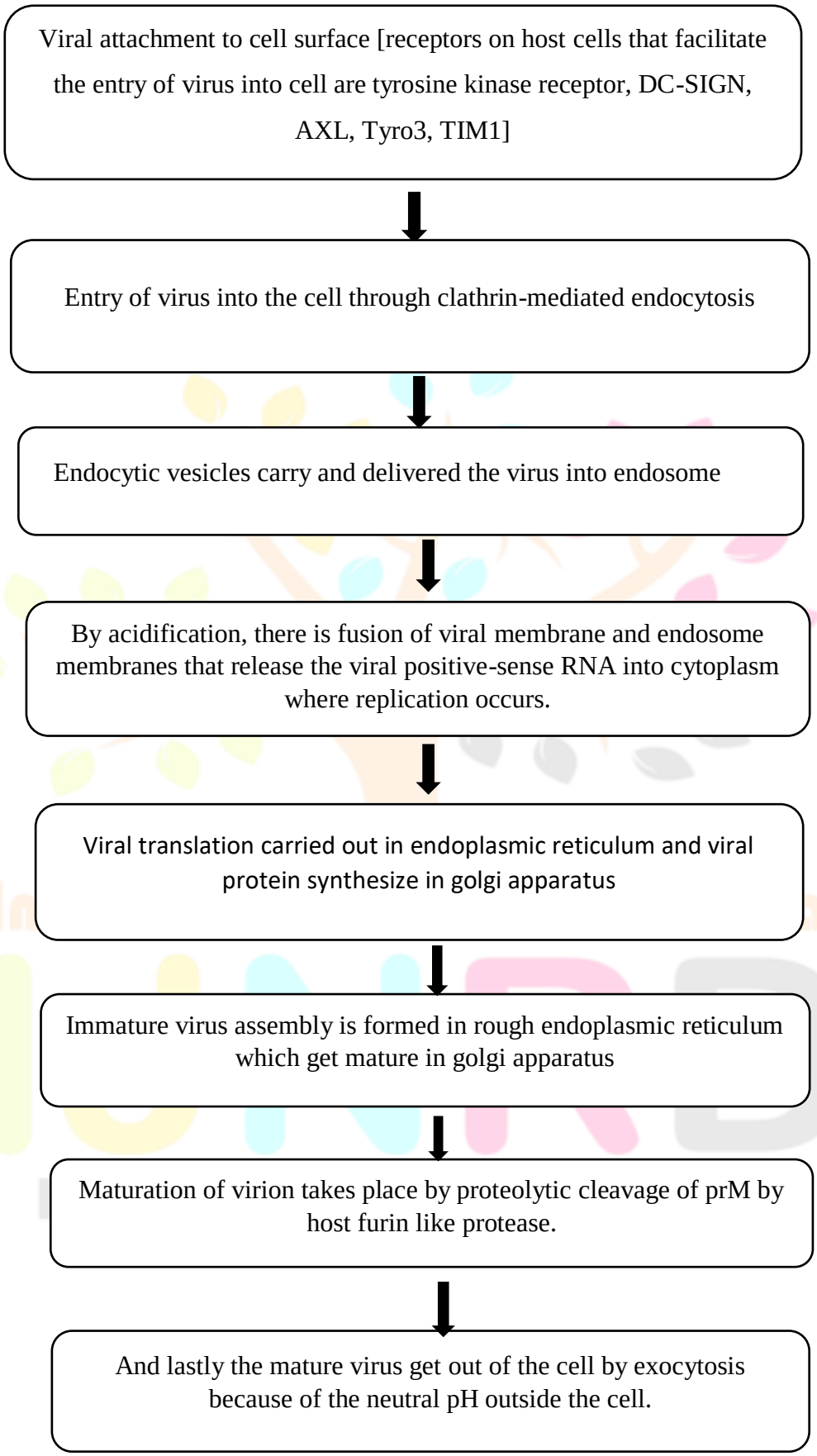
Zika virus infects human embryonic cortical neural progenitor cells (HNPCs) which leads to disruption of cell cycle and promoting cell death (via Capsase-3 mediated apoptosis) and gene dysregulation resulting in cortical thining microcephaly.

Zika virus mainly infect several different types of cells i.e. skin keratinocytes, dermal fibroblast and dendritic cells(DCs). The virus mainly target neuronal progenitor (HNPCs) in the developing brain early infection of zika virus is associated with the proliferation arrest an increase in neuronal progenitor death. Similar results were observed in cortical neurospheres.

The adhesion factors which permit entry of ZIKV are DC-SIGN, AXL, Tyro and TIM-1. zika virus replication activates an antiviral immune response and the production of type-1 interferon in infected cells zika virus infected human primary fibroblast have shown strong upregulation of interferon beta transcripts 24 to 48hrs after infection and milder response have notified for interferon alpha. **Fibroblasts**: zika virus has shown high infection rates in fibroblast 24 to 48hrs after infection. Studies on zika virus infected fibroblast have revealed strong induction of RIG-I and MOA-5 transcripts. RIG-1 and MOA-5 both molecules are capable of initiating signalling process following the detection of intracytoplasmic viral RNA molecules.

Dendritic cells: Zika virus infected dendritic cells which migrate to regional lymph nodes where they stimulate T-cells proliferation, cytokine production and differentiation. Productive infection of dermal fibroblast and DCs cells together with inadequate control of infection by innate and adaptive immune mechanism leads to the zika virus viremia.

Cellular Mechanism



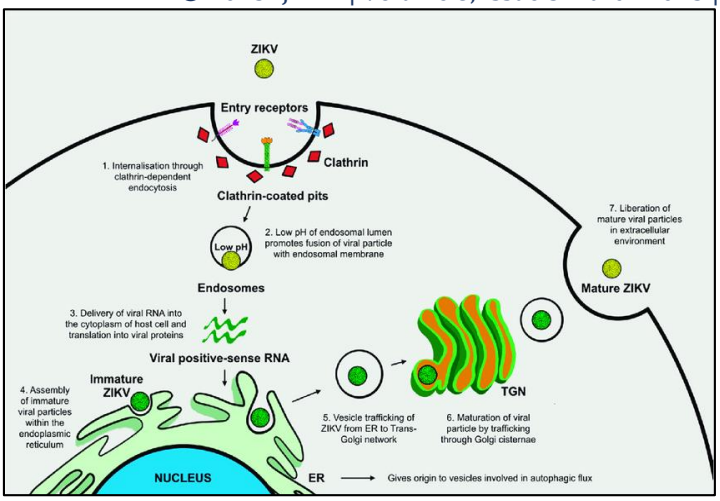


Fig.No.3. Zika Virus Entry mechanism in human cell

Prevention

- Use an Environmental Protection Agency (EPA)–registered insect repellent with active ingredients such as DEET and picaridin.
- Wear long pants and long-sleeved shirts
- Find lodging with air-conditioning or screens to keep mosquitoes outside
- Treat clothes with the insecticide permethrin
- Sleep under a mosquito bed net if you're sleeping outdoors
- Cover a baby's crib, stroller, or carrier with mosquito netting
- Remove stagnant water that may collect in places like planters, buckets, birdbaths, or trash containers.
- Since Zika can be transmitted through sex, the use of condoms can reduce the risk of infection. Zika can be sexuallytransmitted from someone who has no symptoms, so consider whether a sexual partner has lived in or traveled to a place with a high risk of Zika.
- Women who are pregnant or trying to become pregnant should consider avoiding any travel to areas with risk of Zika.

Natural Products

From many years Natural products have been the topic of studies and are the main source of active ingredients which perform important role against most of disease and infection. The creation of innovative antiviral drugs can benefit greatly from the abundance of herbal medicines and purified natural ingredients. In addition to addressing virus-host specific interactions, the identification of the antiviral mechanism from these natural agents has provided insight into how they interact with many stages of the viral life cycle, including viral entry, replication, assembly,

and realease. Following are some natural drugs which can used in the treatment and prevention of zika virus infection which are classified on the basis of their mechanism of action.

1. **Viral Entry Inhibition:**

The entry of ZIKV into the host cell is carried out by various mechanism. It involves different viral-host interactions. Viral entry is common stage of replication cycle can be targeted. These inhibitors can used as a preventive measure. Viral entry is allowed by various receptors present on membrane of host cell.

These inhibitors act by two ways i.e.

i) Direct virucidal effect.

ii) Blocking host cell factors which permit the entry of virus.

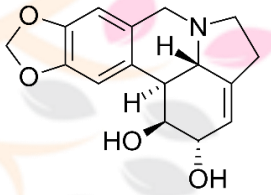
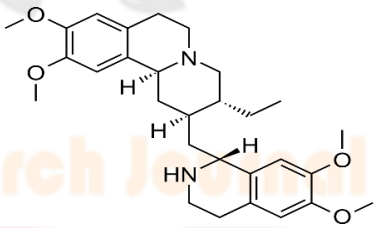
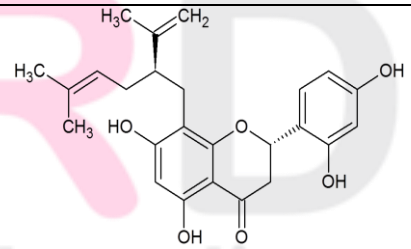
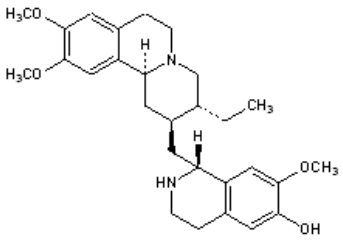
Source	**Family**	**Chemical cnstituents**	**Mechanism of action**
Eucalyptus	Myrtle	Pinocembrin	Inhibits viral RNA and envelope protein synthesis
Digitalis purpurea	Plantaginaceae	Digitonin	Inhibits viral entry
Punica granatum	Lythraceae	Ellagic acid	Reduces cells susceptibility to the virus by binding to host cell surface
Apocynum venetum	Apocynaceae	Isoquercetin	Inhibits viral entry
Streptomyces nanchangensis	Streptomycetaceae	Nanchangmycin	Blocks clathrin-mediated endocytosis, inhibit viral enty
Berberis vulgaris	Berberidaceae	Berberine	Virucidal effect on virus particle
Cephalotaxus drupacea	Taxaceae	Cephalotaxine	virucial effect on virus particle
Vitis vinifera	Vitaceae	Resveratrol	Inhibits a post-entry step and has virucidal effect on virus particle
Rheum rhabarbarum	Polygonaceae	Emodin	Inhibits viral entry and has virucidal effect on virus particles

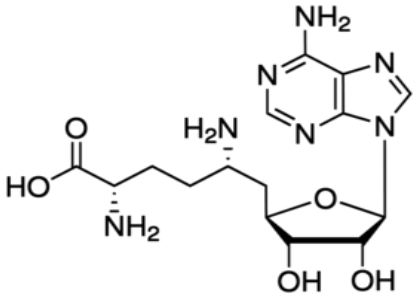
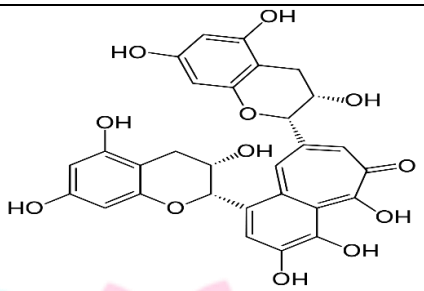
Scutellaria baicalensis and scutellaria lateriflora	Lamiaceace	Baicalein and baicalin	Inhibits a post-entry step and has virucidal effects on virus particles

2 .ZIKV NS5 Protease Inhibitors:

The NS5 protein of ZIKV is largest protein which comprised of two domains, an N-terminal methyltransferase and C-terminal RNA depedent RNA polymerase (RdRp). NS5 protein performs three important roles in viral replication cycle: genome replication, RNA capping and Interferon suppression.

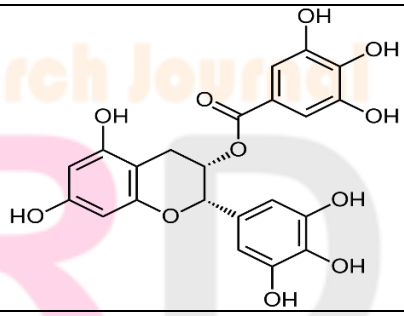
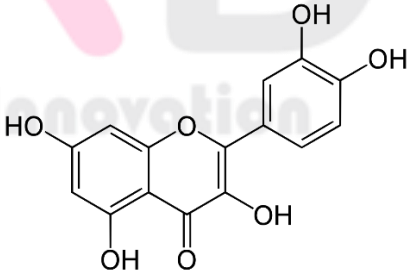
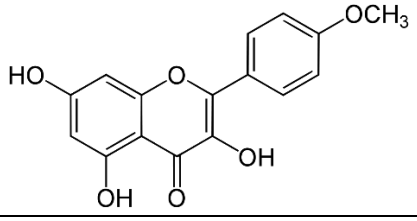
NS5 protease is an ideal target for therapeutic drugs which demonstrated strong inhibitory effect on NS-RdRp-mediated RNA capping activities.

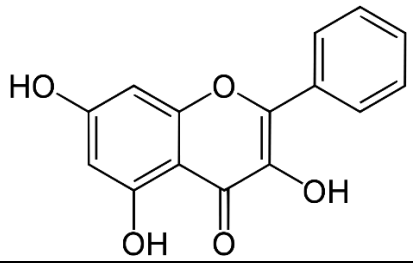
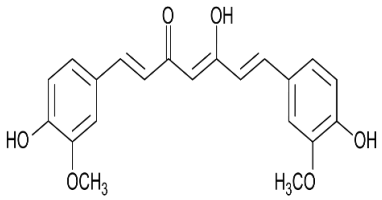
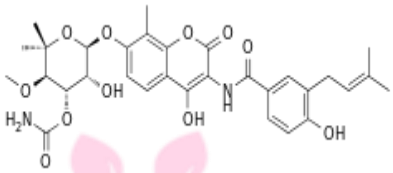
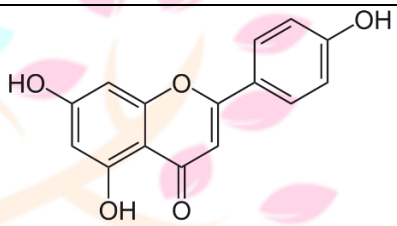
Source	Family	Chemical constituents	Structure
Various species of Amaryllidaceae species	Amaryllidaceae	Lycorine	
Carapichea ipecacuanha	Rubeaceae	Emetine	
Sophora flavescens	Fabaceae	Sophoraflavenone G(SFG)	
Carapichea ipecacuanha	Rubeaceae	Cephaeline	

Streptomyces griseus	Streptomycetaceae	Sinefungin	
Camellia ssinensis	Theaceae	Theaflavin	

3. Inhibitors of NS2B-NS3 Protease:

NS2B is small integral membrane protein, having three hydrophobic domains and a central hydrophillic domain. Central hydrophillic domain plays important role in activation of NS3 protein. And the active NS2B (H)-NS3 protease is important for the cleavage at the different site of connection i.e. NS4A\NS2A, NS2B\NS3, NS3\NS4A, NS4B\NS5. NS2B and NS3 are mainly involved in performing the proteolytic cleavage in the virus for further viral replication.

Source	Family	Chemical constituents	Structure
Green tea	Theaceae	Epigallocatechin gallate (EGCG)	
Onion	Amaryllidaceae	Quercetin	
Kaempferia galangal	Zingiberaceae	Kaempferide	

Alpinia officinarun hance	Zingiberaceae	Galangin	
Curcuma longa	Zingiberaceae	Curcumin	
Streptomyces niveus	Streptomycetaceae	Novobiocin	
Parsley, chamomile, celery,vine-spinach	Apiaceae	Apigenin	

4. Glucose Uptake Inhibitor:

Glucose is the primary source of metabolic energy for the developing placenta and fetus. GLUT1, a membrane-bound protein, is the main glucose transporter across the blood–brain barrier, and the placenta]. A dysfunctional GLUT1 could result in insufficient glucose for normal placental development and functionality, and normal fetal growth, this occurring amidst plentiful glucose in maternal circulation.Zika viral effect on GLUT1 could inhibit access to the glucose needed for normal rapid endothelial growth of the placenta and fetus. GLUT1 deficiency is associated with an increased risk of microcephaly. Additionally, both viral modulations of glucose transporters and microcephaly following intrauterine viral infection.

Phloretin, a glucose transporter inhibitor naturally derived from plants, was used to investigate the glucose dependence of ZIKV replication in host cells. The results showed that phloretin significantly decreased infectious titres of two ZIKV strains, namely MR766 (African genotype) and PRVABC59 (Puerto Rico genotype). The 50% effective concentration (EC50) of phloretin against MR766 and PRVABC59 was 22.85 µM and 9.31 µM, respectively. Further analyses demonstrated that decreased viral production was due to host-targeted inhibition, including decreased apoptotic caspase-3 and -7 activities and reduced phosphorylation of Akt/mTOR pathways. In addition, upon disruption of cellular glucose availability within host cells using 2-deoxy-d-glucose, ZIKV propagation was inhibited.

5. Immune Boosters:

Natural Immunity is capability of our body to defense against any harmful micro-organism or protect from any external factor.

1) The human body has a remarkably sophisticated immune system consisting of white bloods cells and specialized immune molecules that protect the body against invading pathogens.
2) Various micronutrients, vitamins, minerals are key players in stimulating the immune system and dysregulation in all these component can result in impairment of the immune system.
3) Immunostimulants are biologically active substance system of host to fight against various infections.
4) Immunostimulants acts by stimulating cells and activaties like activation of T and B lymphocytes, macrophages, production of cytokines, production of interferon, interleukin, phagocytosis, complement system activity.
5) Naturally occuring compouds derived from plants like glycosides, allkoids, beta-glycan, vitamins, sterols, flavonoids act as natural immunostimulators that many help to combat ZIKV infection.

DRUGS

DRUGS	FAMILY	CHEMICAL CONSTIRUENTS	MECHANISM OF ACTION
Neem (Azadirachta Indica)	Meliaceae	Azadirachtin at lower dose	Stimulates IL-1, INF-Y, TNF-alpha production. T and B cell stimulator.
Tulsi (ocimum sactum	Lemiaceae	Eugenol	Increased levels of IFN-Y, IL-4 and percentage of T-helper cells and NK cells.
Eclipta Prostata	Asteraceae	Whole plant	Increase phagocytic index, increase serum antibody levels, stimulate cellular and humoral immunity.
(Ginger) Zingiber officinate	Zingiberaceae	Gingerol	Stimulation of proliferation of neutrophic, macrophages, lymphocytes.
(Garlic) Allium Sativum	Liliaceae	Alliin, allicin	Enhances macrophage and T lymphocytes, production of INF-Y.
Alove vera	Liliaceae	Barbaloin and alosin	Enhancement of specific and non-specific immune response lysozyme action increase IgM level

(kalmegh) Andrographis paniculate	Acanthaceae	Kalmeghin and andrographolide	Stimulates macrophage migration, proliferation of splenic lymphocytes, stimulation of Ig.
(Shatavari) Asoaragus racemosus	Liliaceae	Asparagamine,racemosol	Stimulation RE system PMN cell
(Haldi) Curcuma longa	Zingiberaceae	Curcumin	Enhanced IgG level, Increased mitogenic response of lymphocytes increase activity of NK cell.

Conclusion

The Zika virus quickly begins to manifest its symptoms in a large number of patients. At that time, a new illness outbreak in Africa caused an increase in public fear. It permeates the majority of the nations, including America, pacific islands, yap islands, and carribean nations. Even though the disease is not associated with significant death rates and the clinical signs of the infection in adult instances are not severe. Infection with the Zika virus can affect prenatal development and result in serious neurological problems. Microcephaly and other serious embryonic brain abnormalities are brought on by the zika virus.

Scientists have uncovered no specific medications or treatments for the condition, according to their studies. WHO asserts that prevention is more crucial than treatment? The WHO believes that prevention is more significant than therapy. The easiest approach to avoid contracting Zika is to avoid being bitten by mosquitoes. According to the article's conclusion, herbal remedies are more vital and effective than allopathic medicines, and they also cause fewer negative effects in humans.

Reference:

1. *Musso D, Gubler DJ. 2016. Zika virus. Clin Microbiol Rev 29:487–524. doi:10.1128/CMR.00072-15*
2. *Byung-Hak song, song-Im Yun, Michael Woolley, Young-Min Lee, 2017. Zika virus: history, epidemiology, transmission and clinical presentation. Journal of Neuroimunology 308.50-64 https://doi.org/10.1016/j.jneuroim.2017.03.001*
3. *Antonio Victor Campos Coelho and Sergio Crovella. 2017. Microcephaly prevalence in infants born to zika virus- infected women: a systematic review and meta-analysis. Int. J. Mol. Sci.18,1714: https://doi.org/10.3390/ijms18081714*
4. *Mariana Baz and Guy Boivin, 2019. Antiviral agent in development for zika virus infections. Pharmaceuticals 12,101, https://doi.org/10.3390/ph12030101*
5. *Mark R. Duffy, D.V.M., M.P.H., Tai-Ho Chen, M.D., W. Thane Hancock, M.D., M.P.H., Ann M. Powers, Ph.D., Jacob L. Kool, M.D., Ph.D., Robert S. Lanciotti, Ph.D., Moses Pretrick, B.S., Maria Marfel, B.S., Stacey*

Holzbauer, D.V.M., M.P.H., Christine Dubray, M.D., M.P.H., Laurent Guillaumot, M.S., Anne Griggs, M.P.H., 2009. Zika virus outbreak on Yap Island, Federated States of Micronesia. The new England journal of medicine 360;24, DOI: 10.1056/NEJMoa0805715

6. *Didier Musso, Claudine Roche, Emilie Robin, Tuxuan Nhan, Anita Teissier, Van-Mai Cao-Lormeau. 2013. Potential Sexual transmission of Zika virus. Emerging infectious diseases 21(2): 359–361. https://doi.org/10.3201%2Feid2102.141363*
7. *David Olagnier, Michela Muscolini, Carolyn B. Coyne, Michael S. Diomond, and John Hiscott, 2016. DNA And Cell Biology 367-372, https://doi.org/10.1089/dna.2016.3404*
8. *Malone RW, Homan J, Callahan MV, Glasspool-Malone J, Damodaran L, Schneider ADB, et al. (2016). Zika Virus: Medical Countermeasure Development Challenges. PLoS Negl Trop Dis 10(3): e0004530. https://doi.org/10.1371/journal.pntd.0004530*
9. *Lowe R, Barcellos C, Brasil P, Cruz OG, Honório NA, Kuper H, Carvalho MS. The Zika Virus Epidemic in Brazil: From Discovery to Future Implications.2018. Int. J. Environ. Res. Public Health, 15(1), 96; https://doi.org/10.3390/ijerph15010096*
10. *Lyle R. Petersen, M.D., M.P.H., Denise J. Jamieson, M.D., M.P.H., Ann M. Powers, Ph.D., and Margaret A. Honein, Ph.D., M.P.H.2016. Zika Virus. N Engl J Med 2016; 374:1552-1563 DOI: 10.1056/NEJMra1602113*
11. *Laith Yakob. Zika Virus after the Public Emergency of International Concern Period,Brazil. 2022. Emerg Infect Dis. 28(4): 837–840.doi: 10.3201/eid2804.211949*
12. *Mariana Baz and Guy Boivin .2019. Antiviral Agents in Development for Zika Virus. Pharmaceuticals, 12(3), 101; https://doi.org/10.3390/ph12030101*
13. *Yuhui Deborah Fong And Justin Jang Hann Chu. 2022.Natural products as Zika antivirals. Med Res Rev.;42:1739-1780: https://doi.org/10.1002/med.21891*
14. *Wang, B., Thurmond, S., Hai, R. et al. 2018.Structure and function of Zika virus NS5 protein: perspectives for drug design. Cell. Mol. Life Sci. 75, 1723–1736, https://doi.org/10.1007/s00018-018-2751-x*
15. *Wahaab A, Mustafa BE, Hameed M, Stevenson NJ, Anwar MN, Liu K, Wei J, Qiu Y, Ma Z.2022. Potential Role of Flavivirus NS2B-NS3 Proteases in Viral Pathogenesis and Anti-flavivirus Drug Discovery Employing Animal Cells and Models: A Review. Viruses; 14(1):44. https://doi.org/10.3390/v14010044*
16. *https://doi.org/10.1016/j.ijantimicag.2019.03.017*
17. *Shah MA, Rasul A, Yousaf R, Haris M, Faheem HI, Hamid A, Khan H, Khan AH, Aschner M, Batiha GE.2021. Combination of natural antivirals and potent immune invigorators: A natural remedy to combat COVID-19. Phytother Res.;35(12):6530-6551. doi: 10.1002/ptr.7228*
18. *Minjee Kim,, Hanul Choi Young Bong Kim.2021.Therapeutic targets and https://doi.org/10.1016/j.ejphar.2021.174144*
19. *Subapriya R, Nagini S. Medicinal properties of neem leaves: a review. Curr Med Chem Anticancer Agents. 2005 Mar;5(2):149-6. doi:10.2174/1568011053174828. PMID: 15777222.*

20. *Motika SE, Hergenrother PJ. Re-engineering natural products to engage new biological targets. Nat Prod Rep. 2020; 37: 1395- 1403. doi:10.1039/d0np00059k*
21. *Yi M, Lin S, Zhang B, Jin H, Ding L. Antiviral potential of natural products from marine microbes. Eur J Med Chem. 2020; 207:112790.*
22. *Rodriguez AK, Muñoz AL, Segura NA, Rangel HR, Bello F. Molecular characteristics and replication mechanism of dengue, zika and chikungunya arboviruses, and their treatments with natural extracts from plants: an updated review. EXCLI J. 2019; 18: 988- 1006.*
23. *Upadhyay SN, Dhawan S, Garg S, Talwar GP. Immunomodulatory effects of neem (Azadirachta indica) oil. Int J Immunopharmacol 1992; 14(7): 1187-93.*
24. *[http://dx.doi.org/10.1016/0192-0561(92)90054-O]*
25. *Makare N, Bodhankar S, Rangari V. Immunomodulatory activity of alcoholic extract of Mangifera indica L. in mice. J Ethnopharmacol 2001; 78(2-3): 133-7.*
26. *[http://dx.doi.org/10.1016/S0378-8741(01)00326-9]*
27. *Momtazi-Borojeni AA, Haftcheshmeh SM, Esmaeili SA, Johnston TP, Abdollahi E, Sahebkar A. Curcumin: A natural modulator of immune cells in systemic lupus erythematosus. Autoimmun Rev 2018; 17(2): 125-35.*
28. *[http://dx.doi.org/10.1016/j.autrev.2017.11.016] [PMID: 29180127]*
29. *Yadav VS, Mishra KP, Singh DP, Mehrotra S, Singh VK. Immunomodulatory effects of curcumin. Immunopharmacol Immunotoxicol 2005; 27(3): 485-97.*
30. *[http://dx.doi.org/10.1080/08923970500242244] [PMID: 16237958]*
31. *Patil A, Kakde MS. Medicinal plant as a natural immunity booster for COVID19- A review. Int J Integr Med 2020; 2(2): 24-7.*
32. *Prakash P, Gupta N. Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian J Physiol Pharmacol 2005; 49(2): 125-31.*
33. *Nirala RK. A review on immsssunomodulatory activity of amla and Aloe vera. Journal of Pharmacognosy and Phytochemistry. 2020;9(5):2014-6.*
34. *Pecora, F.; Persico, F.; Argentiero, A.; Neglia, C.; Esposito, S. The Role of Micronutrients in Support of the Immune Response against Viral Infections. Nutrients 2020, 12, 3198.*
35. *W. P. R. T. Perera, Janitha A. Liyanage, K. G. C. Dissanayake, Hiruni Gunathilaka, W. M. T. D. N. Weerakoon, D. N. Wanigasekara, W. S. K. Fernando, R. M. H. Rajapaksha, R. P. Liyanage, Bingun T. Perera, "Antiviral Potential of Selected Medicinal Herbs and Their Isolated Natural Products", BioMed Research International, vol. 2021, Article ID 7872406, 18 pages, 2021.* https://doi.org/10.1155/2021/7872406
36. *K. A. Szychowski, K. Rybczyńska-Tkaczyk, K. Gaweł-Bęben et al., "Characterization of active compounds of different garlic (Allium sativum L.) cultivars," Polish Journal of Food and Nutrition Sciences, vol. 68, no. 1, pp. 73–81, 2018.*

37. *S. D. Jolad, R. C. Lantz, A. M. Solyom, G. J. Chen, R. B. Bates, and B. N. Timmermann, "Fresh organically grown ginger (Zingiber officinale): composition and effects on LPS-induced PGE2 production," Phytochemistry, vol. 65, no. 13, pp. 1937–1954, 2004.*

38. *V. K. Bharti, J. K. Malik, and R. C. Gupta, "Ashwagandha: multiple health benefits," in In Nutraceuticals: Efficacy, Safety and Toxicity, pp. 717–733, Elsevier, 2016*