ejpmr, 2021,8(1), 115-119

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

CAN *TERMINALIA CHEBULA* (HARITAKI) STOP COVID-19?

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Article Received on 08/11/2020 **Article Revised on 28/11/2020** **Article Accepted on 18/12/2020**

ABSTRACT

Pathogenic microorganisms, such as viruses, bacteria, fungi and other parasites, can cause serious diseases leading to pandemic such as COVID-19 caused by coronavirus. Natural products from medicinal plants may act as suitable weapons to combat such diseases. *Terminalia chebula*, a famous medicinal plant of Bangladesh commonly known as 'Haritaki', was found to be active against viral infections by many investigators. For example, *Terminalia chebula* was shown to be effective against enterovirus (EC_{50} 10.6 μg/mL). In this review work, it is inferred that screening of medicinal plants, including *Terminalia chebula*, against a wide range of viral infections may help to develop new drug against viral pandemics like COVID-19.

KEYWORDS: Haritaki, *Terminalia chebula*, COVID-19, pandemic, new drug development.

1. INTRODUCTION

One of the most dangerous challenges the world is facing today is the COVID-19 pandemic which has caused suffering on an unprecedented scale, with a total of 60 million reported cases and 1.4 million confirmed deaths worldwide as of November, 2020.[1] Diverse courses of action are being explored to treat the infection, many of which are focused on the viral spike glycoprotein (S) and syncytium formation. These are considered as targets of interest due to their role in viral entry and fusion initiated by binding with the Angiotensin Converting Enzyme-2 (ACE-2)[2], and cell death. However, the activity of the spike glycoprotein is not limited to this function only. Another important pathological hallmark of the disease has been identified to be syncytial formation, which has later been investigated to elucidate the probable mechanism.[3,4] The inhibition of such phenomena may be potential approaches to the reduction of the infectivity of the disease. Although many phytoconstituents from medicinal plants have been evaluated against the virus, this particular approach remains unexplored.[5] *Terminalia chebula* is a well-established medicinal plant and available all over the country (**Figure 1**), which has shown promise in the inhibition of syncytial formation by diminishing glycoprotein trafficking[6] and thus, might be effective against the coronavirus. In this review, we have assessed the potential implications of this particular plant in developing therapeutics against the SARS-COV-2.

Figure 1: *T. chebula* tree and fruits.

2. Biosynthesis of Glycoprotein in viral cells

Glycoproteins are molecules that comprise of protein and carbohydrate chains that are involved in many physiological functions including immunity. Many viruses have glycoproteins that help them enter bodily cells, but can also serve to be important therapeutic or preventative targets.

Glycoprotein synthesis is performed sequentially in endoplasmic reticulum and the Golgi apparatus which are important cellular organelles (**Figure 2**). Proteins are modified both co-translationally and post-translationally through the attachment of carbohydrate moiety. The ribosome contains the mRNA which codes for the proteins and this ribosome is attached to the endoplasmic reticulum. The freshly synthesized protein is brought to the lumen of the endoplasmic reticulum. Then, the glycosyl transferase helps to attach the glycan moiety with protein. The glycosylated proteins are transported to the Golgi apparatus where dissociation of the carbohydrate residues is performed by glycosidase. The final attachment of carbohydrates is carried out to produce the matured glycoproteins which are trafficked to their site of action and ready to perform their expected functions.[7]

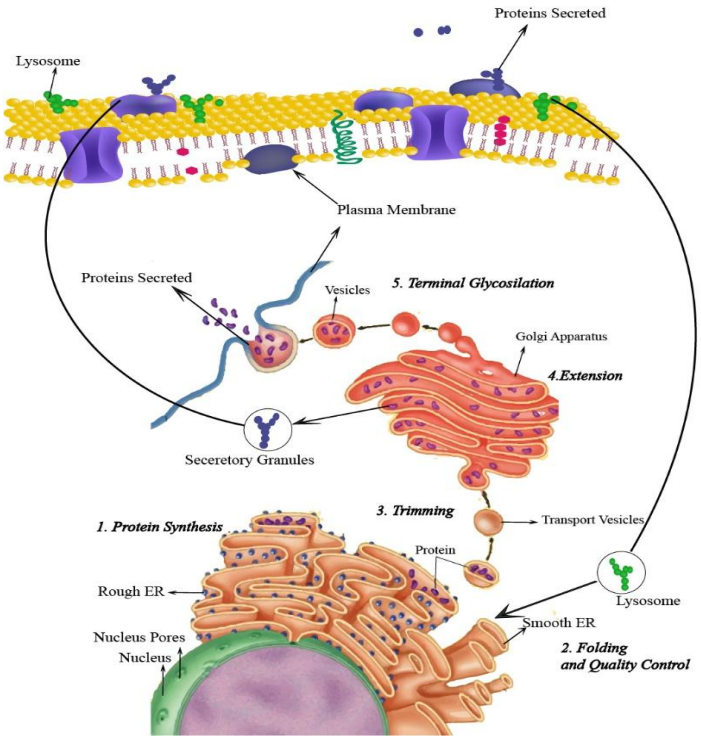

Figure 2: Biosynthesis of glycoproteins.

3. Syncytial formation in SARS-COV-2

A syncytium has been defined as a protoplasmic mass which is the result of fusion of multiple cells, while the individual nuclei remain unfused.[8] The resultant multinucleated cellular mass can originate due to the action of viral fusogenic membrane glycoproteins, leading to cell death.[9,10,11,12] It has been confirmed in a study that the S glycoprotein of the SARS-COV-2 possesses fusogenic properties, which are potentiated following the proteolytic cleavage at the S1-S2 site.[13] This study demonstrated that syncytial formation does not occur if the S protein is not expressed, which suggests that it is the essential glycoprotein for cell-cell fusion (**Figure 3**). Interestingly, even in the absence of proteolytic enzymes, syncytial formation still occurred when the S1-S2 sites were expressed in SARS-S protein. However, in the presence of Trypsin or Transmembrane Protease Serine 2 (TMPRSS2), syncytial formation showed a marked increase. It can, therefore, be inferred from this study that the S protein is responsible for cell-cell fusion even without S1-S2 cleavage, but the cleavage definitely boosts its fusogenic action. Following maturation, a fraction of the S protein does not participate in viral assembly, and is trafficked to the plasma membrane.[14] This migrated fraction is responsible for cell-cell fusion, syncytial formation and eventual cell death.

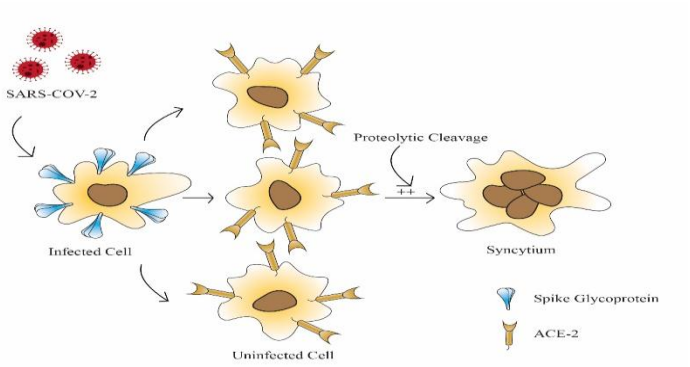

Figure 3: Schematic diagram of syncytium formation induced by viral infection.

A recent histological analysis carried out on SARS-COV-2 infected lungs post-mortem revealed that this feature was present in 36 out of 41 samples (87%)[3] Later on, a study was carried out to elucidate the mechanism of this process using different cell lines.[4] Initially, U2OS cell line expressing the ACE-2 protein was exposed to different concentrations of viral inoculum using a Green Fluorescence Protein-Split Complementation System. It was found that syncytial formation began 6 hours after infection and continued to grow, incorporating neighboring cells. It was found that the GFP+ area increased with the viral load. Much of the fused cells eventually underwent cell death, which was assessed using Propidium Iodide. This study also utilized 293T, A549 and Vero-E6 cell lines, but the phenomenon did not occur in the last two. This suggested that whether syncytial formation will take place after SARS-COV-2 infection, depends on the cell type. This study also confirmed the potentiation effect TMPRSS2 has on this process.

4. Antiviral potential of *Terminalia chebula*

Lee *et al.* showed that the physiological changes of Newcastle disease virus (NDV) in baby hamster kidney (BHK) cell are similar to those of the human immunodeficiency virus (HIV) in that syncytium are formed after infection. HIV infection in vitro induces syncytium formation by cell-to-cell fusion.[6,16]

The antiviral activity of methanolic extract of *Terminalia chebula* bark was tested on Newcastle Disease Virus (NDV) infected Baby Hamster Kidney (BHK) cells.[6] This virus is similar to SARS-COV-2 and human immunodeficiency virus (HIV) as they are capable of producing syncytia following the infection. NDV-Hemagglutinin-Neuramidase protein synthesis was evaluated using hemagglutination in chicken RBCs exposed to infected cells. No significant decrease in hemagglutination units was observed, suggesting that the extract did not notably diminish synthesis of glycoproteins, which have segments extending extracellularly and play a role in cell-cell interactions (**Figure 4**). The cell surface expression of said protein was measured through the hemadsorption percentage, which dropped from 100% to below 50% at a dose of 200 µg/mL.[6] This suggests that glycoprotein trafficking to cell membrane is effectively inhibited by the extract (**Figure 5, black**). Moreover, it was previously established that Nonactin, a naturally occurring cyclic ionophore known as the macrotetrolide antibiotics, hampers the glycoprotein trafficking in the same model, with the generation of oxidative stress, an imbalance between free radicals and antioxidants.[15] Interestingly, the bark extract of *T. chebula* displayed its antibacterial activity against *Bacillus subtilis*, which was weakened in the presence of antioxidants Dithiothreitol and Superoxide Dismutase, an enzyme that helps break down potentially harmful reactive oxygen species (ROS) in cells.

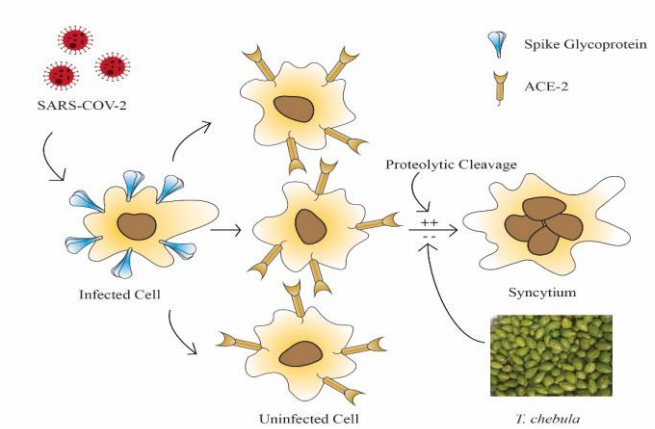

Figure 4: Schematic representation of inhibition of syncytium formation by *T. chebula*.

It can therefore be inferred that *T. chebula* extract acts comparably to Nonactin, where induction of oxidative stress may play a role. This eventually leads to inhibition of syncytial formation (**Figure 5**). Hemagglutination units (HAU, indicated by blue columns in **Figure 5**) was not significantly decreased at any concentration up to 50 µg/mL methanol extracts, on the other hand, Hemadsorption (HAD, indicated by black columns in **Figure 5**) decreased depending on the extract concentration, indicating that *T. chebula* extract blocks the cell surface expression of NDV-HN glycoprotein in a dose-dependent manner.[6] These results collectively imply that the methanolic extract of *T. chebula* has the ability to inhibit the cell surface expression of NDV-HN, but has no visible effects on its synthesis.

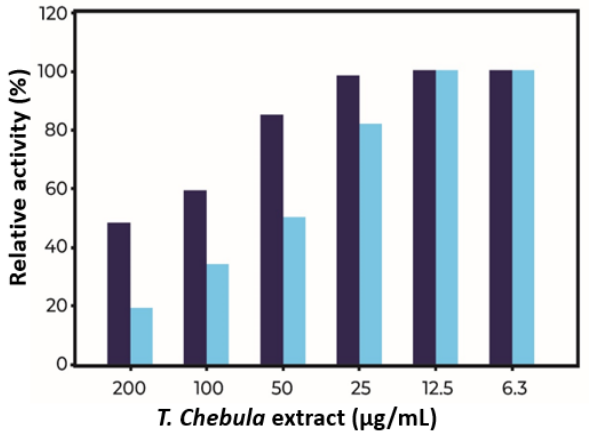

Figure 5: Inhibition of the cell surface expression of Newcastle disease virus-hemagglutinin neuramidase (NDV-HN) glycoprotein by *T. chebula* extract with no significant effects on its synthesis. Blue columns indicate Hemagglutination units (HAU) and black columns indicate Hemadsorption (HAD). Results are expressed as a percentage of the control value (Redrawn by modification from Ref.:6).

Several investigators have investigated the antiviral activity of T. chebula of different viral species such as Herpes simplex virus-2 (HSV-2),[17] hepatitis B virus (HBV),[18] HSV-1, Adenovirus type 5, Echovirus type 11, Measles virus, Rotavirus,[19] and influenza A virus (IAV)[20]

Ajay Kesharwani *et al.* studied the Anti- Herpes simplex virus-2 (HSV-2) activity of *Terminalia chebula* extract and its constituents, chebulagic and chebulinic acids and showed that T. chebula extract, chebulagic and chebulinic acids have higher direct antiviral activity against HSV-2 and efficacy to prevent virus attachment and penetration to the host cells as compared to acyclovir.[17] Kim *et el.* studied the antiviral activities by examining a cell culture system using a hepatitis B virus (HBV) of four medicinal plants (*Terminalia chebula* Retz., *Sanguisorba officinalis* L., *Rubus coreanus* Miq. and *Rheum palmatum* L.) and found that of the four investigated medicinal plants, Terminalia chebula showed the most prominent anti-HBV activities.[18] Ziai *et al.* investigated the antiviral activity against HSV-1, Adenovirus type 5, Echovirus type 11, Measles virus and Rotavirus of twenty-five medicinal plants available in Iran and observed that three plants extract, named *Aristolochia maurorum*, *Terminalia chebula* Retz. and *Cichorium intybus* L. showed significant antiviral activity against HSV-1 and adenovirus type 5 at a concentration nontoxic to the cell lines used.[19] Li *et al.* studied the effect of two compounds, chebulagic acid and chebulinic acid isolated from *T. chebula* on the influenza A virus (IAV) and observed that chebulagic acid and chebulinic acid can effectively inhibit IAV replication. These compounds act as neuraminidase inhibitors and show antiviral potency to both wild-type and oseltamivir-resistant IAV strains.[20]

5. CONCLUSION

The medicinal plant *T. chebula* has established antiviral activity particularly against viral infections with characteristics similar to that of COVID-19. Therefore, further investigation for isolation of the active compound, elucidation of the biochemical mechanism of trafficking inhibition and *in-vivo* analysis is definitely warranted. This may be a promising approach to the development of new antiviral drugs which will diminish the infectivity of the SARS-COV-2.

Author Contributions

MSA has conceived the original idea and designed the outlines of the study. EH, AK, RR, and TA wrote the draft of the manuscript. MSA has taken the photographs (Figure 1). EH (Figures 3,4 & 5) and AK (Figure 2) have drawn and redrawn the figures of the manuscript. SK, AAC, JAC, MRT, EH and AK performed the literature survey. All authors improved the manuscript through mutual discussion by several offline and online meetings. All authors have meticulously gone through the draft and approved the final manuscript before submission.

Funding

This research was performed by self-funding.

Conflicts of Interest

The authors declare no conflicts of interest.

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