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REVIEW ARTICLE

Biological Activities of *Rhazya stricta* Against Influenza Virus

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Abstract

Background: The natural products have played a major role in combating diseases including the viral diseases. *Rhazya stricta* Decne belongs to the family Apocynaceae and found abundantly in various parts of Pakistan. The plant is known for its use in the folk medicine system for the treatment of various diseases. The wide spectrum of the biological properties of the plant include antiviral, antidiabetic, antifungal, antibacterial and anticancer. Over 250 chemical constituents have been reported from the plant including over 100 alkaloids from different parts of the plant. Several alkaloids and non-alkaloid have also shown a variety of biological activities including the antiviral activities. Several active chemical constituents have been reported from different part of the plant. The extracts of the plant have also shown activities against influenza virus. As influenza viruses have developed resistance to available therapies to cope with the virus, it may become another threat like the coronavirus pandemic. Under the circumstances, new therapies are badly needed to meet the growing challenge of the viruses.

Material and Method: The extracts of different parts of the plant were subjected to various bioassays to determine their biological activities. The computer-simulated experiments are becoming one of the effective methods used in drug discovery; enabling the simulation of drug-biological target dynamics, prediction of drug efficacy and design of novel drug molecules within a virtual environment. The extracts of the leaves of the plant were investigated for their antiviral activities *in vitro* to find out their effectiveness against the viral diseases including the influenza viruses and also to identify promising chemical constituents '*In silico*'. The extracts were subjected to *in vitro* experiments by using Madin-Darby Canine Kidney cell line (MDCK) as a substrate for the influenza virus and estimating the inhibition performance of the extracts of the leaves of the plant. The '*In silico*' screening was also carried out to determine the antiviral activities of the plant. The extract of the leaves of the plant was subjected to the MTT say to determine its cytotoxicity as well as antiviral activity against influenza virus. The extract of the leaves of the plant was also subjected to time-of-addition assay to determine mechanism of action of the extract during the life cycle of the virus. The '*In silico*' studies

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analyses were performed, including the molecular docking, drug-likeness analysis, and toxicity risk assessment were conducted to find out state the lead compounds to be developed into an antiinfluenza virus drug. The extract of the plant may prove effective remedy against the influenza virus and may be used to develop drug to deal with the influenza viruses.

Results: The extracts of various parts of *Rhazya stricta* have shown antiviral properties in *in vitro* and *in vivo* experiments. The extracts of the plant have shown potent activity against influenza virus. Keeping in view the potential of the plant against influenza virus, it may be further investigated for its use in treating the viral diseases including the influenza virus and also to develop an effective drug to control the viral diseases including the influenza virus. The '*In silico*' experiments were performed to find out toxicity and antiviral and anti-influenza therapy of the extracts of the leaves of the plant. The doses of the extract of the leaves of the plant at 184.6 µg/ml and 19.71 µg/ml proved to be 50% cytotoxicity concentration and 50% inhibition concentration, respectively. The time of addition assay showed that the extract of the leaves of the plant exerted its activity in the late step of the influenza virus replication cycle. In comparison to Oseltamivir, the lead compounds showed better binding affinity and may be developed into oral drugs with low toxicity risk. Isolation and purification of the lead compounds and testing their antiviral activity *in vitro* and *in vivo* are required.

Conclusion: The extracts of *Rhazya stricta* have shown strong antiviral activities and can effectively treat the coronavirus and influenza viruses. The researches lead to the conclusion that the extracts of the plant could be used as a source for modern and effective antiviral agents and help threatened mankind in the viral resistance battle. The *in vitro* study followed by computational investigations revealed that the extract of the leaves of the plant possess the antiviral potential. The extracts of the leaves of the plant also showed their effectiveness in treating the influenza virus. Further researches, including the *in vitro* and *in vivo* studies are required to analyze the extracts of the plant and its constituents for their antiviral activities.

Introduction

Influenza remains one of the major public health concerns because it causes annual epidemics and can potentially instigate a global pandemic [1]. Numerous countermeasures, including vaccines and antiviral treatments, are in use against seasonal influenza infection; however, their effectiveness has always been discussed due to the ongoing resistance to antivirals and relatively low and unpredictable efficiency of the influenza vaccines compared to other vaccines. The growing interest in vaccines as a promising approach to prevent and control influenza may provide alternative vaccine development options with potentially increased efficiency. In addition to currently available inactivated, live-attenuated, and recombinant influenza vaccines on the market, novel platforms such as Virus-Like Particles (VLPs) and nanoparticles, and new vaccine formulations are presently being explored. These platforms provide the opportunity to design influenza vaccines with improved properties to maximize quality, efficacy, and safety. The influenza vaccine manufacturing process is also moving forward with advancements relating to egg- and cell-based production, purification processes, and studies into the physicochemical attributes and vaccine degradation pathways. These experiments will contribute to the design of more stable, optimized vaccine formulations guided by contemporary analytical testing methods and through implementation of the latest advances in the field.

Influenza is a viral respiratory infection in humans. It is caused by the influenza-A virus (*Alphainfluenzavirus*), influenza B virus (*Betainfluenzavirus*). Influenza viruses C and D (*Gammainfluenzavirus* and *Deltainfluenzavirus*) are also known. The symptoms of influenza virus infection range from a mild upper respiratory tract infection with fever, sore throat, cough and fatigue to severe and in some cases, lethal pneumonia caused by the influenza virus or secondary bacterial infection of the lower respiratory tract. Each year, influenza infects approximately 10% - 20% of the world's population, resulting in 3-5

million hospitalizations, about 290 000-650 000 respiratory deaths and an estimated annual economic burden of $87.1 billion in the United States alone [2-4].

While vaccination is certainly the most preferred approach for the influenza prevention, it is linked with various rates of protection due to inadequate absorption, mismatches with prevalent influenza virus strains, long manufacturing times in chicken eggs and within-season loss of effectiveness [5]. Therefore, antivirals that specifically target the influenza virus functional or structural proteins are also critical for preventing or treating influenza infections. Antivirals against influenza are important in the treatment of hospitalized or critically ill influenza patients, as well as in the early phases of the pandemic when a compatible vaccine is unavailable [6].

Discussion

Resistance of influenza virus to amantadine and the adamantane analogous drug rimantadine was quickly identified and shown to be associated with a single amino acid change at one of five sites in the M2 protein. Due to the development of resistance, adamantanes are no longer effective against influenza type-A [7]. The present gold standard for antiviral treatment of influenza includes various types of Neuraminidase (NA) blockers, such as oseltamivir (tamiflu), zanamivir, and peramivir. NA inhibitor-resistant influenza strains have emerged earlier as a result of drug treatment, emphasizing the need for the discovery of potential drugs targeting different viral gene products [8].

Rhazya stricta is a member of the alkaloid-rich Apocynaceae family. Around 100 indole-type alkaloids have been isolated from its parts, and it is one of the most economically valuable medicinal plants found throughout arid South Asia and the Middle East. Leave extracts were traditionally used to treat a variety of ailments [9,10]. It has been reported that the alkaloidal compounds of the plant possess multiple activities, including antitumor, antimicrobial, and antihypertensive [11]. The extract of the leaves of the plant have shown significant antiviral activity including the activity against the influenza virus.

The influenza viruses have developed resistance to the current classes of drugs, which means they could eventually become more virulent and cause more mortality. The extracts of the leave of *Rhazya stricta* were investigated for their antiviral activity [12]. The study was performed *in vitro* by utilizing the Madin-Darby Canine Kidney (MDCK) cell line as a substrate for the influenza virus and estimating the inhibition performance of the extract of the leaves of the plant [11].

The '*In silico*' screening was conducted to explore the antiviral activity of the phytochemicals of the plant. The cytotoxicity of the extract of the leaves of the plant was studied besides its antiviral activity against influenza virus (A/ Puerto Rico/ 8/ 34 (H1N1)) using the MTT assay. The mode of action of the extract of the leaves of the plant during the viral life cycle was tested by using the time-of-addition assay. *In silico* analyses were performed, including molecular docking, drug-likeness analysis and toxicity risk assessment to state the leading compounds to be developed into an anti-influenza virus drug. The 50% cytotoxicity and inhibition concentrations of the extract of the leaves of the plant was found as 184.6 µg/ ml and 19.71 µg/ ml, respectively.

The time of addition assay revealed that the extract of the leaves of the plant exerted its activity in the late step of the influenza virus replication cycle [11]. In comparison to oseltamivir, the leading compounds showed better binding affinity and can be developed into oral drugs with low toxicity risk. The authors suggested that further studies are required to isolate and purify lead compounds and

determining their *in vitro* and *in vivo* antiviral against the influenza virus.

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