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Berberine as a new neuraminidase inhibitor drug: A systematic review

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ARTICLE INFO	ABSTRACT
Article Type: Review *Article History:* Received: 5 April 2023 Accepted: 11 August 2023 *Keywords:* Antivirus agents Influenza Natural products Viral neuraminidase Allosteric binding site	Influenza neuraminidase (NA) plays a main role in the viral replication of the influenza virus. It has been considered one of the targets for anti-influenza drugs. Anti-influenza drugs such as zanamivir, oseltamivir, and peramivir can fight the virus via the inhibition of NA. However, due to adverse reactions, the resistance of the viral strains and sudden changes in NA inhibitors, the identification of novel inhibitors is needed. Nature products such as berberine have been reported against influenza. In this systematic review, we have focused on the anti-influenza effects of berberine and its main role in the inhibition of NA of the virus. For this aim, "Berberine" was searched with "Influenza" or "flu" or "common cold" or "neuraminidase" in Web of Science, PubMed, Scopus, and Google Scholar databases from 1990 to April 2023. Studies have demonstrated that berberine and its derivatives have a wide range of biological effects such as antiviral effects against viruses like herpes simplex virus, human cytomegalovirus, and influenza A virus. The present study indicates that berberine and some of its derivatives are able to inhibit the influenza virus through NA blockade. Berberine is able to superimpose into the allosteric binding site and shows reversible non-competitive behavior in the ligand-receptor interaction for the inhibition of NA.

Introduction

Influenza as an infectious disease has serious effects on health and human life (1-3). It has caused annual influenza epidemics with significant morbidity and mortality (4). Annually, influenza epidemics result in three to 5 million cases of severe sickness and cause about 290 000 to 650 000 deaths worldwide (5,6).

Influenza viruses are from the Orthomyxoviridae family. The genome of these viruses contains negative-sense RNA segments that encode viral proteins. The envelope of the influenza virus consists of two main surface glycoproteins, neuraminidase (NA) and hemagglutinin (HA) (Figure 1). HA is necessary for receptor binding and for the fusion of the virus and the host membrane. NA cleaves the terminal sialic acid residues on cell surfaces and facilitates the

release of the virus from host cells. NA plays a main role in the release and spreading of virions. Also, some studies have shown the role of NA in facilitating HA-mediated fusion and in the virus invasion stage of the human airway epithelium (7,8).

At present, there are three classes of anti-influenza viral drugs: 1) NA inhibitors (NAIs) such as peramivir, zanamivir, laninamivir, and oseltamivir; 2) matrix-2 proton channel inhibitors (M2) such as rimantadine and amantadine, and 3) antiviral PA endonuclease inhibitor such as baloxavir marboxil. NAIs are well tolerated and are effective in the replication of both influenza viruses (A and B) and thus could be a major target for anti-influenza drug development (9,10). NAIs are used clinically worldwide; however, synthetic NAIs have shown

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Research Highlights

- Influenza is an infectious disease that has been caused by annual influenza epidemics with significant morbidity and mortality.
- Influenza neuraminidase (NA) plays a main role in viral replication, spread, and pathogenesis of the Influenza virus.
- NA inhibitors (NAIs) have been developed as a major target for anti-influenza drugs.
- The alkaloids such as berberine have been considered as a resource for production herbal drugs against influenza viruses.
- Berberine inhibits various strains of influenza virus by blocking of viral NA subunit.
- Molecular docking on berberine based derivatives has been revealed that an allosteric and non-competitive site may have been associated with the molecule-inhibitor interaction.

Figure 1. A schematic of the Influenza virus.

a few adverse reactions such as abdominal pain, diarrhea, vomiting, and nausea. Also, the emergence of resistance in viral strains to some NAIs has been reported previously (11,12). These challenges have caused many researchers to focus on the development of new NAIs from natural products. In this regard, natural products can be used to discover new anti-influenza drugs (13).

Previous studies that have widely evaluated polyphenols, flavonoids, alkaloids, glucosides, and saponins indicated that these natural products can be able to block adherence, penetration, and replication in influenza viruses.

Alkaloids have a wide distribution in the plant kingdom. They are found in a lot of families such as Loganiaceae, Menispermaceae, Papaveraceae, Leguminosae, and Ranunculaceae families (14). Alkaloids have indicated various biological effects such as analgesic, anti-inflammatory, and anticancer properties (15-17). Moreover, alkaloids as a main source of phytochemicals can be used for the development and discovery of new natural anti-influenza drugs. Alkaloids with their inhibitory effects on the replication of influenza viruses and inhibition of inflammation in the lungs can be considered resources for the production of herbal drugs against influenza viruses (18,19). Moradi et al have indicated that the anti-influenza A virus activity of *Peganum harmala* L. extract might be attributed to its alkaloid components (20). Also, alkaloids can inhibit the NA in the influenza virus and improve the disease. Kim et al, based on screens for the identification of novel NA inhibitors, evaluated the NA inhibitory activity of *Corydalis turtschaninovii* rhizome extract. The extract had an inhibitory effect equal to 68% at 30 µg/mL. Also, they evaluated 10 isoquinoline alkaloids isolated from the plant. Berberine as an isoquinoline alkaloid indicated stronger NA inhibitory activity (21). In other studies, berberine has been shown to exert antiviral effects against viruses such as herpes simplex, human cytomegalovirus, and influenza A (22,23). This review has been focused on berberine and its effects as a natural alkaloid that can exert anti-influenza effects and inhibit the virus via targeting the NA protein.
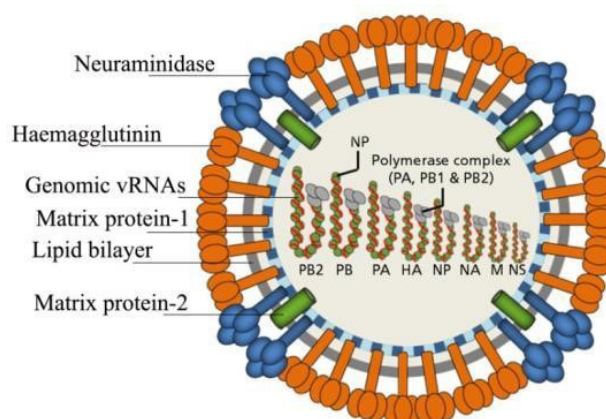
Search strategies
Preliminary searches
In this systematic review, "Berberine" was searched with "Influenza" "flu" "common cold", or "neuraminidase" in scientific literature databases, such as Web of Science (21 articles with duplicates) and PubMed (30 articles with duplicates) via Endnote software. Also, other sources such as Scopus (32 articles with duplicates), and Google Scholar were searched (139 articles with duplicates). The articles published between 1990 and April 2023 were searched.

Piloting of the study selection process
The duplicated articles and articles without an abstract (in English) were excluded. The retrieved articles from the databases were then analyzed. At first, the abstracts were reviewed based on predefined inclusion and/or exclusion criteria.

Formal screening of search results against eligibility criteria
In this stage, full texts were retrieved to assess study eligibility. The articles that addressed the effects of berberine and its derivatives on the Influenza virus were selected, and the papers that exerted anti-influenza effects and/or inhibited virus via targeting the viral NA protein were focused. Figure 2 is the study flowchart to show how the articles were selected for this systematic review.

Results and Discussion
Berberine, an isoquinoline with a range of biological activities
Berberine, an isoquinoline derivative alkaloid (Figure 3), is a bitter-tasting alkaloid called yellow root that is extracted from plants used in traditional medicine from various countries such as Iran, China, India, and America. It is a bright yellow-colored alkaloid and is a chief alkaloid found in fruits, stems bark, and even roots of *Berberis* species, especially *Berberis vulgaris*. Between
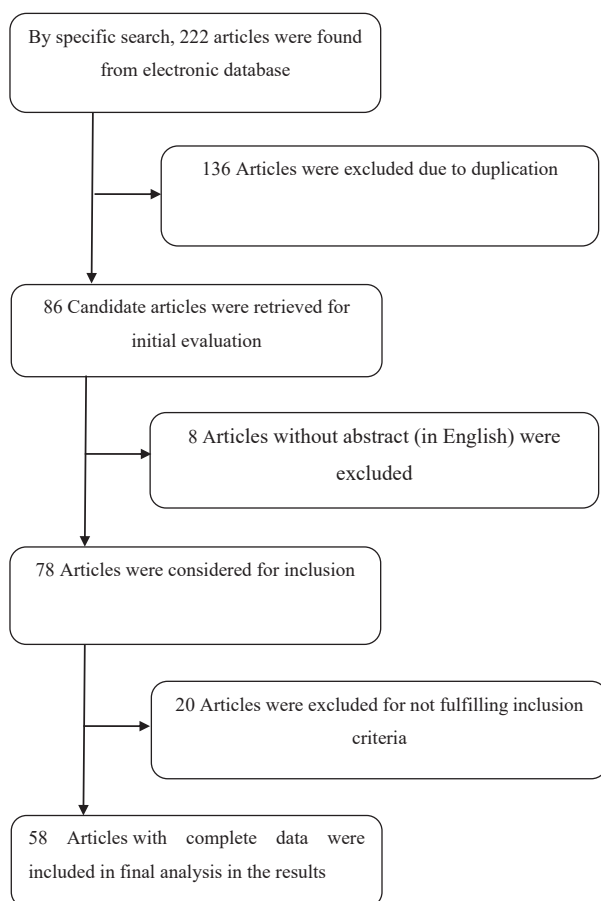
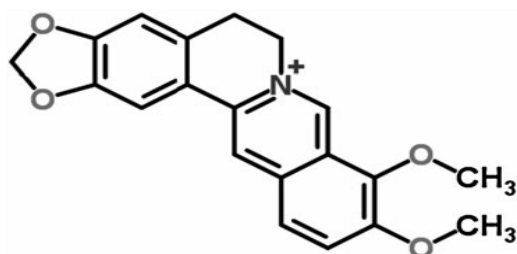**Figure 2.** Flowchart of the process of analyzing the articles.

Figure 3. Berberine structure.

Chinese herbs, *Berberis sargentiana, Coptis chinensis, and Phellodendron amurense* are their primary sources. Also, it is isolated from the roots or rhizome of *Cortex phellodendri, Rhizoma coptidis, Berberis aristata, Berberis petiolaris, Berberis aquifolium, Berberis thunbergii, Berberis asiatica,* and *Hydrastis canadensis* (24-27).

Berberine is relatively soluble in the water. It has demonstrated a range of biological activities such as antimicrobial, anti-inflammatory, antioxidant, anticancer, neuroprotective, antidepressant, and hepatoprotective effects. It has also been applied to treat hypertension, trachoma, acute diarrhoea, and oriental sores (28-32). Berberine as a potent antioxidant improves diabetes and protects against nephropathy and other metabolic syndrome-associated kidney diseases (33-35). The antioxidant properties and anti-inflammatory effects of berberine cause a decrease in the negative symptoms in neuropathic patients (36,37). Studies have shown that berberine has also a potent effect on lung damage and other pulmonary inflammations (38-40). Furthermore, berberine has been shown to have antiviral effects against viruses such as herpes simplex, human cytomegalovirus, and influenza A (41-43). In vitro and in vivo studies have indicated that HB-13, as an active compound of berberine derivatives, has a strong anti-HSV activity (44,45).

Antiviral effects of berberine on influenza viruses
The study results of Botwina et al showed the strong anti-influenza properties of berberine in vitro and ex vivo. This study revealed that berberine could block the transport of viral ribonucleoproteins to the cytoplasm by blocking the influenza-induced ERK/MAPK signaling pathway (46). In addition, Yan et al evaluated the effects of berberine, isolated from *Coptis chinensis* Franch, on the influenza virus. Their results showed that berberine suppresses the viral replication in cell culture and in the lungs of mice and alleviates viral infection-induced lung injury significantly. The study indicated that berberine inhibits the initiation of virus-induced T-cell responses and suppresses the expression of TLR7/NF-κB signaling (47).

The preclinical studies have indicated that berberine and its derivatives can inhibit various strains of influenza virus by blocking of viral NA subunit. In a study by Wu et al in 2007, berberine has suppressed the cytopathic effects (CPE) of MDCK cells induced by influenza virus and has reduced NA activity in vitro (44). The CPE method in MDCK cells showed that berberine could exhibit obviously antiviral activity. The NA assay further demonstrated the anti-influenza effects of berberine. It showed that the viral NA activity was dramatically reduced in the concentrations of 0.00625–0.05 g/L of berberine. Also, when influenza virus was injected to mice, berberine decreased virus titers in the lungs, prolonged the mice's mean survival duration, and reduced mortality. Moreover, berberine has relieved pathological changes in mice lungs. The results indicated that berberine could directly have antiviral activities on the infection of the influenza virus in vitro and in vivo (22). Berberine, as a main bioactive compound of *Coptis chinensis* rhizome, had a higher affinity to six selected proteins than commercial drugs such as oseltamivir, zanamivir, and natural binding ligand sialic acid to inhibit NA (48).

Furthermore, berberine analogs have been shown inhibitory effects on the influenza virus. In the Enkhtaivan et al study, the biological activities of substituted piperazine-based berberine analogs, conjugated through a pentyloxy side chain, have been analyzed. All the analogs were screened for their in vitro antiviral activities against some influenza strains. These compounds were found to

be potential NA inhibitors via merging with the active site of NA (49).

In addition, in an evaluation of synthesized berberine derivatives on influenza virus particles, the synthesized berberine derivatives (BD-1, BD-2, BD-5, and BD-19) inhibited NA activity more than basic berberine. Especially, BD-5 revealed antiviral activity higher than the commercial NA inhibitor drug Oseltamivir. This derivate of berberine could bind to viral NA subunits better than Oseltamivir. BD-5 was docked exactly to the pocket of NA and bound to the important residues of NA activity. Additionally, BD-5 showed drug-likeness properties (11). Kumar and colleagues also examined several berberine–benzothiazole derivatives (BBDs) for their anti-influenza activities on various strains of the virus. They indicated that BBDs had a potent antiviral activity and were able to block viral NA activity. They also evaluated the influence of the binding ability of BBDs with the virus via a molecular docking study. The results revealed that BBDs exhibited an inhibitory effect on the NA of influenza viruses via the attachment of ligand and NA active site residues (50).

Probable mechanisms of NA inhibition by berberine

NA is an essential enzyme for releasing progeny viruses from the surface of an infected host cell. So, Influenza viruses aggregate at the cell surface when NA is inactivated (51). One of the main functions of NA is to cleavage sialic acid from the surface of the cell. Sialic acid, as a cell receptor to which the influenza virus attaches through the HA protein, presents on some cell surface proteins such as the viral glycoproteins. The sialic acids on HA and NA cleavage as the proteins move to the cell surface through the secretory pathway. Also, NA can act during the entry of influenza virions into the epithelial cells of the respiratory tract. The epithelial cells of the respiratory tract contain many sialic acid-containing glycoproteins that are bathed in mucus. When influenza virions are trapped in the mucus of the respiratory tract, they will bind to sialic acids. Interaction between sialic acid and the virus will prevent from binding them to a susceptible cell. When the influenza virions attach to a cell, they bind to receptors containing the sialic acid and are rapidly taken into the cell before the NA can remove the carbohydrate from the surface of the cell (52).

Among available drugs, Relenza (zanamivir) and Tamiflu (oseltamivir) are structurally similar to sialic acid. They tightly bind to the active site of the NA enzyme (52). Whenever they bind to NA, newly synthesized viruses remain immobilized because NA cannot cleavage sialic acids from the cell surface and virions cannot spread from one cell to another cell. We think berberine inhibits viruses via this mechanism (Figure 4). Amino acid residues in or near the active site of NA may have effects on inhibitor binding (53).

Wang and Wade analyzed a series of NA-inhibitor complexes via combined models. They indicated that differences in inhibitory activity on NA are commonly determined via interactions with 12 residues in the active site and 1 bound water molecule. So, strong inhibitors should have structural features that make interactions with these protein residues desirable. They indicated that in the active site of NA, a negatively charged group is desirable for binding to the pocket formed via Arg118, 292, and 371, and a positively charged group is needed in the Arg156 and Asp151 pockets. There are two hydrophobic regions in which hydrophobic groups can be introduced to enhance binding intensities: one is created by Arg224, Ile222, and Trp178, and the other one is formed through rotating Glu276 (Figure 5) (54).

In another study by Sahoo et al, the interaction profile of 13 natural ligands, with the active site residues of NA receptors by forming hydrogen bonds drawn by LigPlot, has been shown. Among natural ligands, theaflavin found in green tea has been docked with minimum binding energy by forming hydrogen bonds with the amino acid residue of the receptor. Theaflavin was found to interact
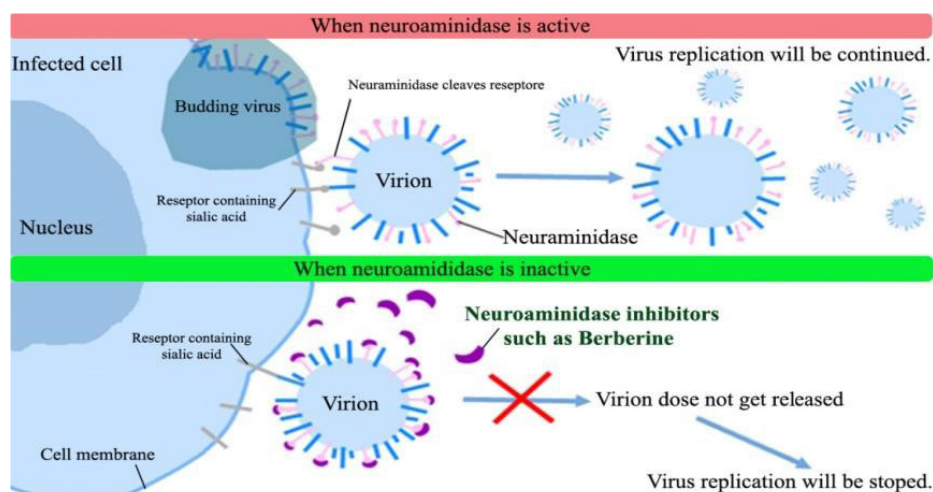

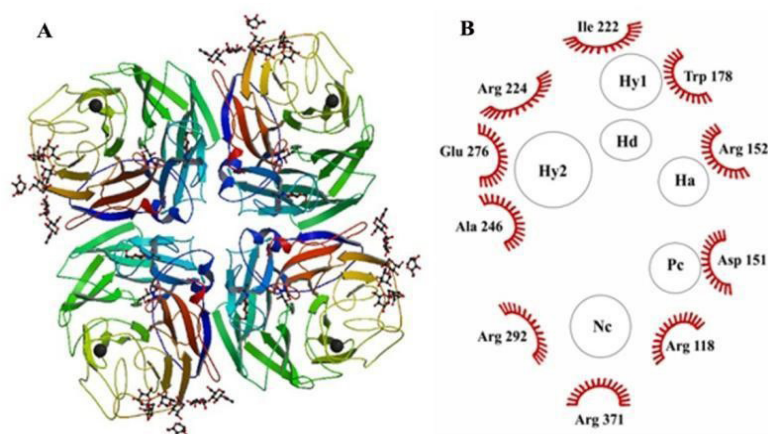**Figure 4.** The effects of neuraminidase inhibitors such as berberine.

Figure 5. Native influenza virus neuraminidase and diagrams of corresponding NA residues (54).

with the amino acid residues like Arg430, Asn344, Asp199, Arg193, Asp 152, Asp151, and Arg118 of NA by creating hydrogen bonds (Figure 6) (55).

Also, in another study, molecular modeling for NA in complex with a novel antiviral compound (PDBID 4MJU) resulted in satisfactory accommodation of the studied alkaloids in the hydrophobic binding site of 4MJU that was mainly packed between the residues Glu 432, Lys 430,
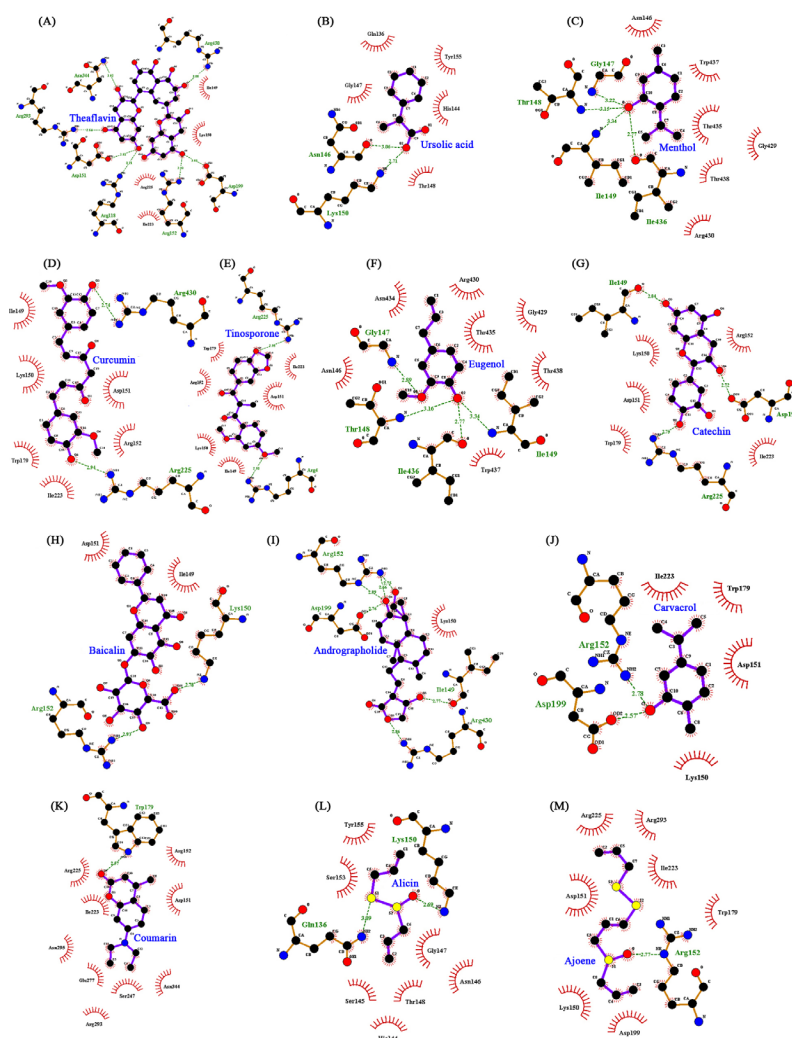

Figure 6. Interaction profile of 13 natural ligands with the active site residues of NA receptors. The Creative Commons Attribution Non-Commercial License (55).

Arg 371, Asp 151, and Arg118 (56). Moreover, molecular docking on isoquinoline alkaloids such as berberine has revealed that an allosteric and non-competitive site may have been associated with the molecule-inhibitor interaction (55,56). Actually, the quaternary isoquinoline alkaloids have shown reversible non-competitive behavior in the ligand-receptor interaction for the inhibition of NA. Moreover, based on the optimal position of the ligands, the location of binding was predicted to be an allosteric site. Based on this information, the residues of amino acids for hydrogen bonds and p–p stacking interactions with two ligands, i.e., the positively charged HIS 346 and negatively charged amino acids ASN 417 in the binding site, have provided new data for the development of NA inhibitors with non-competitive sites. In addition, the best binding pose of berberine into the NA has been exclusively evaluated. Berberine was superimposed into the allosteric binding site and exhibited p–p stacking with TRY320 and HIS346 (21).

A pilot molecular docking study for the evaluation and confirmation of NA inhibitory by berberine

In a pilot study by the authors of this paper for evaluation and confirmation of the NA inhibitory activity of berberine, we evaluated the NA inhibitory activity of berberine via molecular docking by the AutoDock software and then LigPlot+. We observed that berberine superimposed into the binding site with Glu385 and Asn448 by forming hydrogen bonds. It interacted with the amino acids such as Tyr320, Asn432, Ile416, His346, Gly404, Thr400, Ala402, Asn401, Asn386, Arg384, Val383, Gku369, Tyr370, Phe362, and Leu371 of NA through forming hydrophobic bonds (Figure 7). In this interaction, the estimated free energy of binding was -8.24 kcal/mol, and the estimated

inhibition constant was 911.12 nM.

Since the function of NA is related to its structure, binding berberine into the NA *via* specialized amino acid residues can interfere with the protein function (57).

Safety of berberine

Berberine is possibly safe at recommended doses. It is relatively nontoxic for most adults when it is taken by mouth or applied to the skin for short-term use. However, it has side effects such as hypotension at high doses. It may cause cardiac damage, gastrointestinal discomfort, and flu-like symptoms. Moreover, it may keep the liver from removing bilirubin fast enough. Also, drug interactions have been reported with tetracyclines. It should be used with caution during pregnancy and breastfeeding as it has been shown to affect bilirubin metabolism in infants (58). Pharmaceutical researchers have to consider these side effects in designing new anti-influenza drugs.

Conclusion

The present study indicates that berberine and some of its derivatives are able to inhibit various strains of influenza virus by blocking the viral NA subunit. Berberine, via superimposing into the allosteric binding site of the influenza virus, shows reversible non-competitive behavior in the ligand-receptor interaction for the inhibition of NA. These results can be considered for discovering an anti-influenza agent that affects influenza viruses through NA blockade.

Authors' contribution

Conceptualization: Javad Saffari-Chaleshtori, Mohammad-Taghi Moradi.
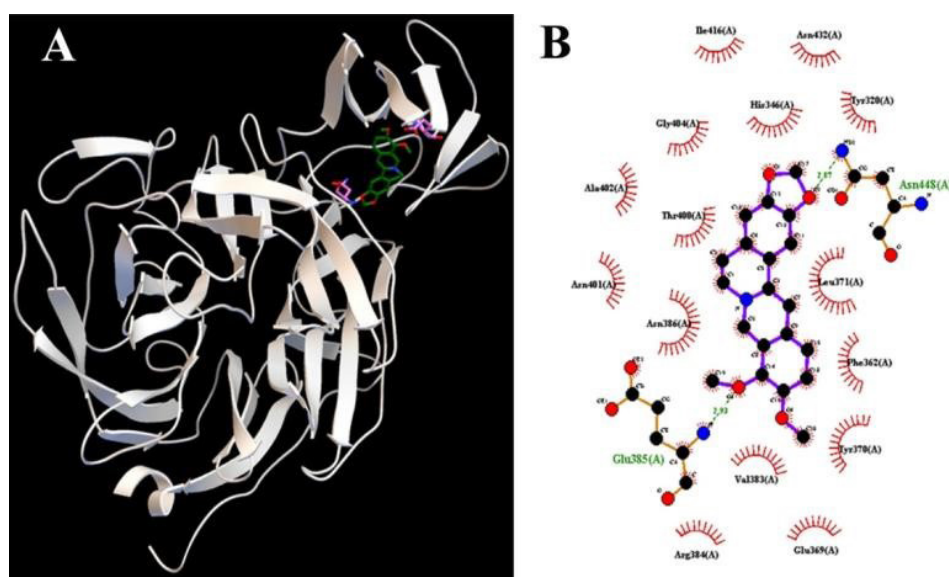**Data curation:** Majid Asadi-Samani, Javad Saffari-Chaleshtori, Mohammad-Taghi Moradi.

Figure 7. The best binding poses of berberine into the neuraminidase.

Formal analysis: Dhiya Altememy, Javad Saffari-Chaleshtori, Mohammad-Taghi Moradi.

Funding acquisition: Majid Asadi-Samani, Dhiya Altememy, Javad Saffari-Chaleshtori, Mohammad-Taghi Moradi.

Investigation: Majid Asadi-Samani, Dhiya Altememy, Javad Saffari-Chaleshtori, Mohammad-Taghi Moradi.

Methodology: Majid Asadi-Samani, Javad Saffari-Chaleshtori, Mohammad-Taghi Moradi.

Project administration: Majid Asadi-Samani, Mohammad-Taghi Moradi.

Resources: Majid Asadi-Samani, Dhiya Altememy, Javad Saffari-Chaleshtori, Mohammad-Taghi Moradi.

Software: Javad Saffari-Chaleshtori, Mohammad-Taghi Moradi.

Supervision: Majid Asadi-Samani, Mohammad-Taghi Moradi.

Validation: Majid Asadi-Samani, Dhiya Altememy, Javad Saffari-Chaleshtori, Mohammad-Taghi Moradi.

Visualization: Majid Asadi-Samani, Mohammad-Taghi Moradi.

Writing–original draft: Majid Asadi-Samani, Javad Saffari-Chaleshtori, Mohammad-Taghi Moradi.

Writing–review & editing: Majid Asadi-Samani, Dhiya Altememy, Javad Saffari-Chaleshtori, Mohammad-Taghi Moradi.

Conflict of interests
No potential conflict of interest was reported by the authors.

Ethical considerations
Authors have carefully monitored ethical issues such as text plagiarism, duplicated publication, misconduct, data fabrication, and falsification.

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