Iranian J Virol 2017;11(2): 29-35
©2017, Iranian Society of Virology. All rights reserved.

Original Article

The Antiviral Effects of Curcumin Nanomicelles on the Attachment and Entry of Hepatitis C Virus

Naseri S[1], Darroudi M[2], Aryan E[1], Gholoobi A[3], Rahimi H R[3], Ketabi K[1], Movaqar A[1], Abdoli M[1], Gouklani H[4], Teimourpour R[5], Meshkat Z[1*]

1. Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Nuclear Medicine Research Center (NMRC), Ghaem Hospital, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Department of Modern Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
4. Molecular Medicine Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
5. Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.

Abstract

Background and Aims: Hepatitis C virus (HCV) is a member of the Flaviviridae family, which causes approximately 500,000 deaths annually. HCV infection treatment is often associated with significant adverse effects. Curcumin is an active ingredient of turmeric which has therapeutic anti-inflammatory effects in many diseases including infectious ones. Although curcumin is not soluble in water, if it is synthesized in the form of nanomicelles, it will be water soluble and can be absorbed in the gastrointestinal tract (GI). In this study, the antiviral effects of curcumin nanomicelles were investigated on the attachment and entry of HCV particles.

Materials and Methods: The cytotoxicity of curcumin nanomicelles was determined in Huh7.5 cells and their antiviral effects on the attachment and entry of HCV was investigated in a cell culture system.

Results: Curcumin nanomicelles could decrease the viral load in the cell culture supernatants compared to virus control.

Conclusions: According to the results of this research, we determined the antiviral effects of curcumin nanomicelles in the later stages of HCV replication.

Keywords: Hepatitis C virus, Curcumin, Herbal medicines, Nanomicelles.

Introduction

Hepatitis C is an infectious disease of the liver, which is caused by hepatitis C virus (HCV). HCV is a member of the family Flaviviridae (1), consisting of several genotypes. Genotypes 1 and 4 of this virus were found to be more resistant to treatment than genotypes 2 and 3. The most important drugs that are used to treat HCV infection are Ribavirin and peginterferon. But, only about 40 to 50% of patients with genotype 1 infection can get cured. The new approved drugs for treatment of this infection are Telaprevir, Paritaprevir, Boceprevir, and Simeprevir, which inhibit NS3/4A protease. Daclatasvir inhibits NS5A and Sofosbuvir inhibits NS5B (2-4). As herbal medicines are safe, effective, and inexpensive; they have attracted many attentions (5).

[*]**Corresponding author:** Zahra Meshkat: Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +98-5138012453, Fax: +98-5138002960, E-mail: meshkatz@mums.ac.ir.

Previous investigations have shown that there are some herbal medicines, which have anti HCV effects including Milk Thistle, Glycyrrhizin, Kampo, Phyllanthus, Cinnamon, and Curcumin (6-14).

Curcumin is a yellow substance derived from the rhizome of Turmeric which has a lot of benefits such as being antioxidant and anti-inflammation. Curcumin is able to bind some metals such as iron and copper (15). According to series of studies, curcumin has an anti-microbial effect on different kinds of bactria and viruses including HIV, Influenza virus, Herpes simplex virus-1 and 2, Coxsackievirus, Hepatitis B virus, HCV, Human Papillomavirus, and Japanese encephalitis virus (16-24).

Nanomicelles are self-assembled nano-sized (10 to 100 nm) particles that can be used as drug carriers for various therapies. Their structure consists of a hydrophilic core and a hydrophobic shell (25). One study showed that curcumin nanomicelles have anti-HTLV-1 effect (26).

The goal of this study was to evaluate the antiviral effects of curcumin nanomicelles on the attachment and entry of HCV infection in cell culture.

Methods

Cell culture and virus reproduction. In this study, JFH1 vector harboring HCV genotype 2b isolate and its appropriate cell line for propagation (Huh7.5) were used to produce HCV particles. JFH1 vector and Huh7.5 cells were provided under an MTA agreement [ACADEMIC MATERIAL TRANSFER AGREEMENT (MTA 1832, APP1025 pJFH1, APC49 Huh7.5 cells)] between Apath, L.L.C., a limited liability corporation of the State of Missouri and Mashhad University of Medical Sciences (MUMS). As previously described (27), Huh7.5 cells were cultured in DMEM/ high glucose (Biosera, France) supplemented with 10% of FBS (Gibco, the USA) and 1% of Penicillin and Streptomycin antibiotics (Caisson, the USA). When the confluency of the cells reached about 70 %, the residual media were removed and cells were inoculated by viruses. After 48-72 hr of incubation, the supernatants containing viruses was collected.

MTT Assay. MTT Assay was used to determine cytotoxicity of curcumin nano-micelles and peginterferon asa control drug. Briefly, Huh7.5 cells were cultured in a 96 well plate; each well contained about 10000 cells. The plate was incubated at 37ºC with 5% CO2 for 24 hr. Eight 2-fold serial dilutions of peginterferon were made from 180 mg/mL stock solution (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128).

For curcumin nanomicelles, three concentration of 0.256, 0.128, and 0.032 mg/mL were made. After removing media, different concentrations of peginterferon and curcumin nanomicelles were added to wells. Each concentration of either treatment was tested in triplicate.

Then, the plate was incubated for 24 hr. The media of the wells was removed. Subsequently, 100 microliter of new media plus 10 µl of MTT reagent was added to each well. After 4 hr of incubation at 37ºC under 5% of CO2, 100 microliters of DMSO was added to each well. Eventually, the optical density was read at 570 and 630 nm by an ELISA reader.

IC50 Determination. The concentration induced 50% cytotoxicity (IC50) was calculated using Microsoft Office Excel and CalcuSyn software (Biosoft, version 2/1).

Exposing the virus to peginterferon and curcumin nanomicelles

Approximately, 10000 cells were seeded in each well and the plate was put in an incubator at 37ºC under 5% of CO2 for 24 hr. Fifty µl of the virus and 200 µl of 1 IC50 of either peginterferon or curcumin nanomicelles were mixed and kept at 4ºC. After discarding the media, the mixtures of the virus with peginterferon and/or curcumin nanomicelles were added to the wells. Eventually, the supernatant of each well was collected after 48 hr and was used save for RNA extraction.

RNA extraction and Real Time PCR. RNA extraction was performed using Genet Bio kit (South Korea). Then, Real Time PCR was done by Iranian Noving Gene kit (Iran) using Corbett Rotor Gene.

Results

Cell culture. Figure 1 shows Huh7.5 cells attached to the flask bottom as monolayer epithelial cells.

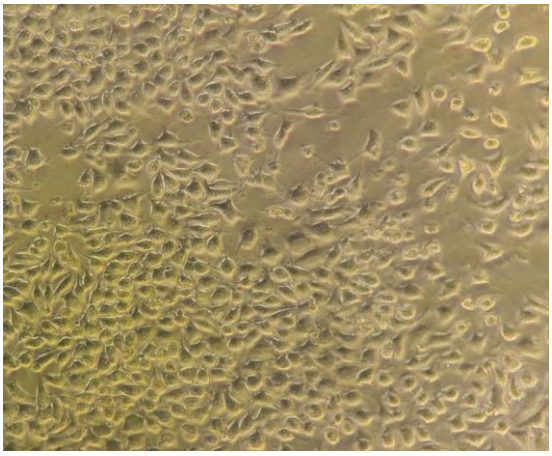


Fig. 1. Hhu7.5 cells. The image was taken by an inverted microscope with 100 X magnification.

MTT assay and IC50 determination. The percentage of the viable cells against different concentration of peginterferon and curcumin nanomicelles was determined using MTT assay.

Chart 1 shows the viability of Huh7.5 cells following exposure to different concentrations of peginterferon. The highest concentration was 0.18 mg/mL.

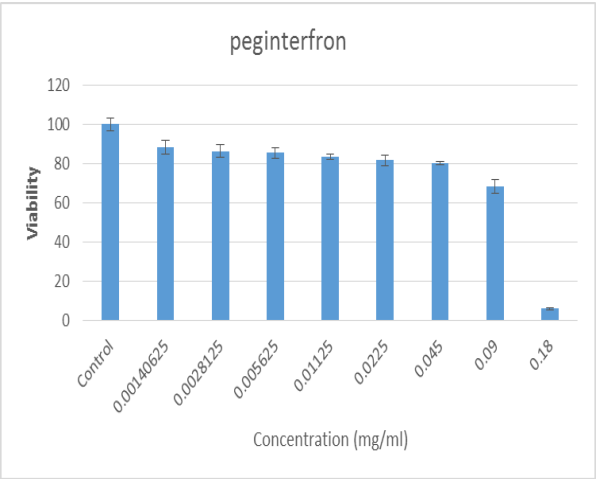


Chart. 1. Cytotoxicity effect of peginterferon on Huh7.5 cell line.

Chart 2 shows the viability of Huh7.5 cells against different concentrations of curcumin nanomicelles. The highest concentration was 0.256 mg/mL.

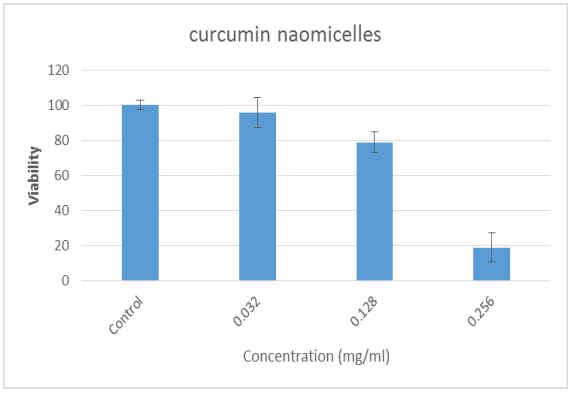


Chart. 2. Cytotoxicity effect of curcumin nanomicelles on Huh7.5 cell line.

The IC50 values of peginterferon and curcumin nanomicelles were obtained from the dose response curves (Figures 2 and 3).

.

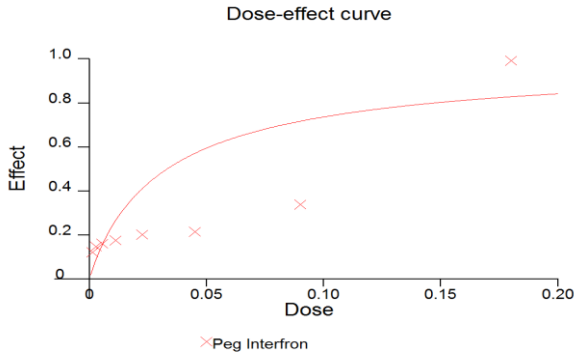


Fig. 2. The dose–effect curve of peginterferon.

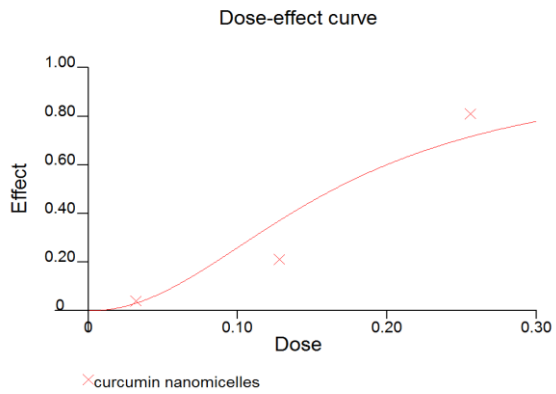


Fig. 3. The dose–effect curve of curcumin nanomicelles.

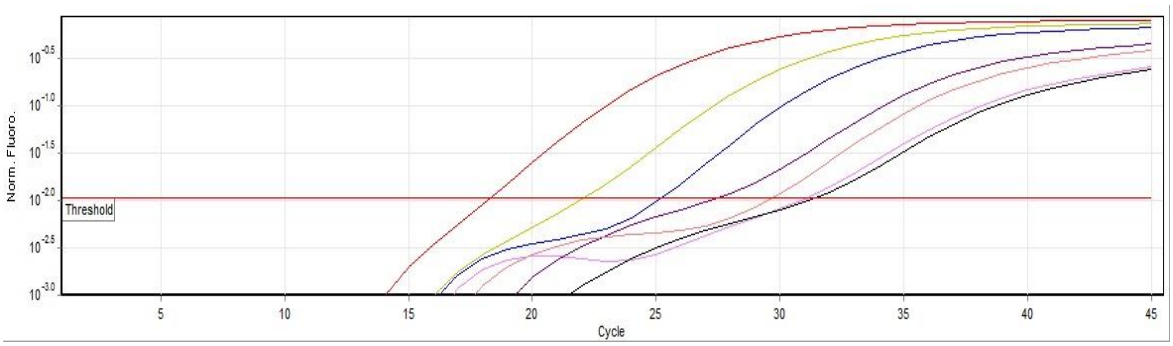


Chart. 3. Sigmoid curve of virus multiplication

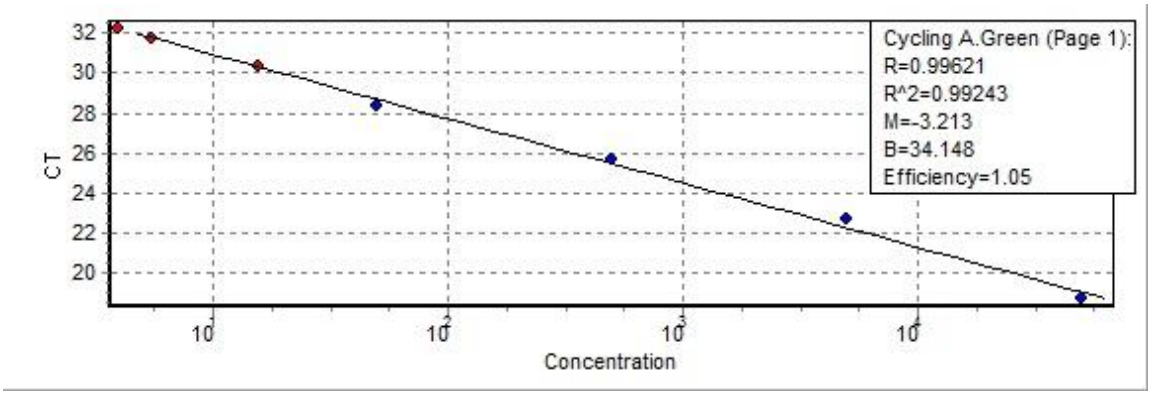


Chart. 4. The standard curve by concentration and CT

The values of IC50 for Huh7.5 cells were achieved as 0.03298 mg/mL and 0.1647 mg/mL in the cases of peginterferon and courcumin nanomicelles, respectively.

Chart 3 shows the Sigmoid curves of virus multiplication and chart 4 illustrates the standard curve based on the concentrations against CT values. According to Real Time PCR results (R=0.996, Efficiency= 1.05), the viral load of virus control and virus in combination with peginterferon, and curcumin nanomicelles were 1250, 4000 and 1000 IU/mL, respectively.

Discussion

HCV is one of the infectious agents that causes liver cirrhosis and liver cancer (28). More than 185 million people (2.8 % of the world population) were estimated to be infected by this virus. About 500000 people died due to this infection annually. About 75 % of patients with acute HCV infection develop chronic infection and 20 % of them develop liver cirrhosis after 2 decades (29). A study was done in Mashhad in which 67% and 33% of HCV-infected patients were women and men, respectively. Generally, less than one percent of people from Mashhad are infected by HCV (30). Data from a systematic review showed that 0.16 percent of Iranian population are infected by HCV (31). The number of people infected with HCV in the world continues to rise and it is predicted that about 3 million people will be infected by this virus annually. Although this virus is endemic in the world, yet in some regions the number of patients is different. Africa, Southeast Asia, and Latin America have the highest genotypes variations and North America, European countries, and Australia have the lowest genotypes variations (32).

The most important medicines that are used for the treatment of this virus are peginterferon and Ribavirin. But only 40-50 % of people with HCV genotype 1 can be cured (33). In 2011, new medications with the name of direct-acting antivirals (DAA) were developed

and represented a breakthrough in HCV treatment. They have more than 90% response to treat most of the genotypes. The duration of treatment is between 12 to 24 weeks (34).

There are new FDA approved medicines for HCV treatment such as Telaprevir, Paritaprevir, Boceprevir, and Simeprevir which inhibit NS3/4A protease. Daclatasvir inhibits NS5A and Sofosbuvir inhibits NS5B (2-4). The safety of Simeprevir is generally acceptable. Some of the most adverse effects of peginterferon and Ribavirin are fatigue, influenza-like illness, pruritus, headache, and nausea. Patients taking simeprevir might cause increased frequency and severity of hyperbilirubinemia (35).

Some adverse effects of Sofosbuvir were reported when it was combined with peginterferon and/or longer treatment occurred including fatigue, headache, nausea, insomnia, pruritus, irritability, anemia, asthenia, and diarrhea. When this medicine was combined with Ribavirin and peginterferon, the adverse effects on appetite, influenza like illness, pyrexia, chills, neutropenia, and myalgia were decreased. Daclatasvir is generally tolerated well and the most prevalent adverse effects of it are headache, fatigue, nausea, and diarrhea (34). The other disadvantage of DAA can be their expensiveness. For instance, the cost of taking Sofosbuvir for 12 weeks is 84000 dollars (36).

Ribavirin is a teratogenic medicine for both men and women. That is the reason for avoiding pregnancy during and 6 months after the end of therapy. Also breastfeeding should not be done. Developing reversible arrhythmia or cardiomyopathy is rare in patients with cardiac problems while they are treating with interferon.

Because death from cardiac failure is more likely to happen in patients who are suffering from both HCV and significant cardiac disease, anti-viral therapy can be dispensed. Other reasons that can stop the use of Ribavirin and peginterferon are hepatic decompensation and renal failure. These medicines can enhance liver failure and monitoring of patients are needed. Renal failure will increase the amount of Ribavirin and peginterferon in patients' serum that may cause hemolysis. The immune system of patients is influenced by these medicines and probably this is the mechanism of their antiviral activities. In HCV patients with comorbid autoimmune diseases, both medicines can make the condition worse.

For this reason, the treatment will be dangerous when the autoimmune disease is not controlled. The most common adverse effects of peginterferon are fatigue, muscle aches and psychological disorders such as depression, irritability, anxiety and sleep disturbance.

The most common adverse effects of ribavirin are hemolysis and anemia. Some other adverse effects that are rare are hearing impairment, hair thinning and loss, insomnia, visual disorders, interstitial pneumonia, pancreatitis, colitis and exacerbation of inflammatory diseases (37).

As herbal medicines are safe, efficient, and cost-effective, using them has attracted many attentions (5). Studies show there are some herbal medicines which have anti HCV effects including Milk Thistle, Glycyrrhizin, Kampo, Phyllanthus, Cinnamon, and Curcumin.

Curcumin is used as a treatment of many diseases including Inflammatory Bowel Disease, Irritable Bowel Syndrome, Postoperative Inflammation, Arthritis, Psoriasis(38), and some Bactria such as Vibrio harveyi, Bacillus subtilis, Aeromonashydrophila, Staphylococcus aureus, Streptococcus agalactiae and some viruses such as HIV, Influenza virus, Herpes simplex virus-1 and 2, Coxsackievirus, Hepatitis B virus, Hepatitis C virus, and Human Papilloma virus, and Japanese encephalitis virus (16-24).

Nanomicelles are self-assembling nanosized colloidal dispersions with sizes between 10 to 100 nm. They have a hydrophobic core and hydrophilic shell. Nanomicelles are used as carriers of hydrophobic medicines as many medicines are hydrophobic (25).

So far, there is few studies about herbal medicines in nanomicelles because most herbal medicines are hydrophilic.

Curcumin nanomicelles have been developed for oral use because they have a high bioavailability compared to the ordinary version of curcumin. After taking capsules, the soft gels of SinaCurcumin opens and transitpasses into the small intestine from the stomach after less than 15 minutes.

Curcumin nanomicelles could be dissolved in unstirred water layer after reaching the small intestine. Unlike curcumin which is insoluble in water, curcumin nanomicelles can be absorbed in GI tract because they are soluble in water. As curcumin is not water soluble, many methods have been suggested to make it water soluble. Preparing curcumin as nanomicelles is one of the most successful methods (39).

A study showed curcumin can decrease the gene expression of HCV via suppression of the Akt-SREBP-1 activation, not by NF-kB pathway. Curcumin has anti-cancer effects against anti-hepatocellular carcinoma (13).

A study by Anggakusuma et al in 2014 showed curcumin could inhibit the entry of HCV, but could not inhibit HCV RNA replication or viral assembly/release in Huh7.5 cells (14).

So far a few studies about anti-viral effects of curcumin nanomicelles have been done,

A study by Mohammadi et al in 2014 showed curcumin nanomicelles could have anti-HTLV-1 effects (14). In our study, this type of curcumin could decrease the viral load of HCV.

Conclusion

After treatment of HCV infected cells with peginterferon and curcumin nanomicelles, it was shown that the viral load of HCV infected cells treated with peginterferon were increased, but this viral load for cells treated with curcumin nanomicelles were decreased.

It is suggested that curcumin nanomicelles can have anti-viral effects on the attachment and entry of hepatitis C infection. Further study on the mechanism of curcumin nanomicelles effect is suggested.

References

1. Manns MP, Buti M, Gane E, Pawlotsky JM, Razavi H, Terrault N, et al. Hepatitis C virus infection. Nat Rev Dis Primers. 2017;3:17006.

2. Ermis F, Senocak Tasci E. New treatment strategies for hepatitis C infection. World J Hepatol. 2015;7(17):2100-9.

3. Feeney ER, Chung RT. Antiviral treatment of hepatitis C. Bmj. 2014;348:g3308.

4. González-Grande R, Jiménez-Pérez M, González Arjona C, Mostazo Torres J. New approaches in the treatment of hepatitis C. World J Gastroenterol. 2016;22(4):1421-32.

5. Saleem TM, Chetty CM, Ramkanth S, Rajan V, Kumar KM, Gauthaman K. Hepatoprotective herbs–a review. Int J Res Pharm Sci. 2010;1(1):1-5.

6. Polyak SJ, Morishima C, Lohmann V, Pal S, Lee DYW, Liu Y, et al. Identification of hepatoprotective flavonolignans from silymarin. Proc Natl Acad Sci U S A. 2010;107(13):5995-9.

7. Rossum TGV, Vulto AG, Hop WC, Brouwer JT, Niesters HG, Schalm SW. Intravenous glycyrrhizin for the treatment of chronic hepatitis C: a double-blind, randomized, placebo-controlled phase I/II trial. J Gastroenterol Hepatol. 1999;14 (11):1093-9.

8. Ashfaq UA, Javed T, Rehman S, Nawaz Z, Riazuddin S. An overview of HCV molecular biology, replication and immune responses. Virol J. 2011;8(1):161.

9. Kayano K, Sakaida I, Uchida K, Okita K. Inhibitory effects of the herbal medicine Sho-saiko-to (TJ-9) on cell proliferation and procollagen gene expressions in cultured rat hepatic stellate cells. J Hepatol.29(4):642-9.

10. Cyong J-C, Kim S-M, Iijima K, Kobayashi T, Furuya M. Clinical and pharmacological studies on liver disease treated with Kampo herbal medicine. Am J Chin Med. 2000;28(03n04):351-60.

11. Ravikumar YS, Ray U, Nandhitha M, Perween A, Raja Naika H, Khanna N, et al. Inhibition of hepatitis C virus replication by herbal extract: Phyllanthus amarus as potent natural source. Virus Res. 2011;158(1):89-97.

12. Li S, Kodama EN, Inoue Y, Tani H, Matsuura Y, Zhang J, et al. Procyanidin B1 purified from Cinnamomi cortex suppresses hepatitis C virus replication. Antivir Chem Chemother. 2010;20(6): 239-48.

13. Kim K, Kim KH, Kim HY, Cho HK, Sakamoto N, Cheong J. Curcumin inhibits hepatitis C virus replication via suppressing the Akt-SREBP-1 pathway. FEBS letters. 2010;584(4):707-12.

14. Anggakusuma, Colpitts CC, Schang LM, Rachmawati H, Frentzen A, Pfaender S, et al. Turmeric curcumin inhibits entry of all hepatitis C virus genotypes into human liver cells. Gut. 2014; 63(7):1137.

15. Hatcher H, Planalp R, Cho J, Torti FM, Torti SV. Curcumin: From ancient medicine to current clinical trials. Cell Mol Life Sci. 2008;65(11):1631-52.

16. Bourne KZ, Bourne N, Reising SF, Stanberry LR. Plant products as topical microbicide candidates: assessment of in vitro and in vivo activity against herpes simplex virus type 2. Antiviral Res. 1999;42(3):219-26.

17. Chen D-Y, Shien J-H, Tiley L, Chiou S-S, Wang S-Y, Chang T-J, et al. Curcumin inhibits influenza virus infection and haemagglutination activity. Food Chem. 2010;119(4):1346-51.
18. Divya CS, Pillai MR. Antitumor action of curcumin in human papillomavirus associated cells involves downregulation of viral oncogenes, prevention of NFkB and AP-1 translocation, and modulation of apoptosis. Mol Carcinog. 2006;45 (5):320-32.
19. Dutta K, Ghosh D, Basu A. Curcumin protects neuronal cells from Japanese encephalitis virus-mediated cell death and also inhibits infective viral particle formation by dysregulation of ubiquitin-proteasome system. J Neuroimmune Pharmacol. 2009;4(3):328-37.
20. Kim HJ, Yoo HS, Kim JC, Park CS, Choi MS, Kim M, et al. Antiviral effect of Curcuma longa Linn extract against hepatitis B virus replication. J Ethnopharmacol. 2009;124(2):189-96.
21. Kim K, Kim KH, Kim HY, Cho HK, Sakamoto N, Cheong J. Curcumin inhibits hepatitis C virus replication via suppressing the Akt-SREBP-1 pathway. FEBS Lett. 2010;584(4):707-12.
22. Si X, Wang Y, Wong J, Zhang J, McManus BM, Luo H. Dysregulation of the Ubiquitin-Proteasome System by Curcumin Suppresses Coxsackievirus B3 Replication. J Virol. 2007;81 (7):3142-50.
23. Sui Z, Salto R, Li J, Craik C, Ortiz de Montellano PR. Inhibition of the HIV-1 and HIV-2 proteases by curcumin and curcumin boron complexes. Bioorg Med Chem. 1993;1(6):415-22.
24. Zandi K, Ramedani E, Mohammadi K, Tajbakhsh S, Deilami I, Rastian Z, et al. Evaluation of antiviral activities of curcumin derivatives against HSV-1 in Vero cell line. Nat Prod Commun. 2010;5(12):1935-8.
25. Vadlapudi AD, Mitra AK. Nanomicelles: an emerging platform for drug delivery to the eye. Ther Deliv. 2013;4(1):1-3.
26. Mohammadi A, Fazeli B, Taheri M, Sahebkar A, Poursina Z, Vakili V, et al. Modulatory effects of curcumin on apoptosis and cytotoxicity-related molecules in HTLV-1-associated myelopathy/ tropical spastic paraparesis (HAM/TSP) patients. Biomed Pharmacother. 2017;85:457-62.
27. Teimourpour R, Meshkat Z, Gholoubi A, Nomani H, Rostami S. Viral Load Analysis of Hepatitis C Virus in Huh7.5 Cell Culture System. Jundishapur J Microbiol. 2015;8(5).
28. Nyalakonda H, Utay NS. A new era of therapy for hepatitis C virus infection. Curr Opin Infect Dis. 2015;28(5):471-8.
29. Wandeler G, Dufour JF, Bruggmann P, Rauch A. Hepatitis C: a changing epidemic. Swiss Med Wkly. 2015;145:w14093.
30. Gerayli S, Pasdar A, Shakeri MT, Sepahi S, Hoseini SM, Ahadi M, et al. Haplotype Analysis of Hemochromatosis Gene Polymorphisms in Chronic Hepatitis C Virus Infection: A Case Control Study. Iran Red Crescent Med J. 2016;18(6):1-7.
31. Alavian SM, Asl MA, Lankarani KB, Shahbabaie MA, Bahrami Ahmadi A, Kabir A. Hepatitis C Infection in the General Population of Iran: A Systematic Review. Hepat Mon. 2009;9(3): 211-23.
32. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis. 2005;5(9):558-67.
33. Vermehren J, Sarrazin C. The role of resistance in HCV treatment. Best Pract Res Clin Gastroenterol. 2012;26(4):487-503.
34. Geddawy A, Ibrahim YF, Elbahie NM, Ibrahim MA. Direct Acting Anti-hepatitis C Virus Drugs: Clinical Pharmacology and Future Direction. J Transl Int Med. 2017;5(1):8-17.
35. Izquierdo L, Helle F, François C, Castelain S, Duverlie G, Brochot E. Simeprevir for the treatment of hepatitis C virus infection. Pharmacogenomics Pers Med. 2014;7:241-9.
36. Rosenthal ES, Graham CS. Price and affordability of direct-acting antiviral regimens for hepatitis C virus in the United States. Infect Agents Cancer. 2016;11:24.
37. Weigand K, Stremmel W, Encke J. Treatment of hepatitis C virus infection. World J Gastroenterol. 2007;13(13):1897-905.
38. Gupta SC, Patchva S, Aggarwal BB. Therapeutic Roles of Curcumin: Lessons Learned from Clinical Trials. AAPS J. 2013;15(1):195-218.
39. Rahimi HR, Nedaeinia R, Sepehri Shamloo A, Nikdoust S, Kazemi Oskuee R. Novel delivery system for natural products: Nano-curcumin formulations. Avicenna J Phytomed. 2016;6(4): 383-9