**MEDICINAL PLANTS AS ALTERNATIVE ANTI- HCV DRUGS**

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**Introduction**

Hepatitis is an inflammation of the liver. The liver is an essential organ that processes nutrients, filters the blood, and fights infections. When the liver is inflamed or damaged, its function can be affected. It is caused due to variety of non-infectious agents like heavy alcohol consumption, toxins, some medications, and certain medical conditions. However, infectious virus often causes hepatitis. There are five main strains of Hepatitis virus, referred to as types A, B, C, D, and E. They all cause liver disease, while they differ in important ways including modes of transmission, severity of the illness, geographical distribution and prevention methods. Hepatitis may range from acute and chronic infection. In particular, types B and C leads to chronic disease in hundred millions of people and the most common cause of liver cirrhosis, liver cancer and viral hepatitis-related deaths.

For Hepatitis A and B, there is effective vaccine available, while there is no vaccine for Hepatitis C infection. Most patients infected with HCV infection get rid of the virus themselves, but in most cases, the acute stage of HCV infection may lead to a chronic stage of infection. Since there is no vaccine available for HCV, antiviral therapy is suggested. In the late 2000s, FDA has approved antivirals such as a combination of Ribavirin and pegylated Interferon-$ α$(IFN-$α$) which were effective initially, owning to drastic side effects that have been exhibited is an escalating global health issue. These new drugs include direct-acting antiviral (DAA) agents that specifically block a viral enzyme or function protein, and host-targeted agents (HTA) that blocks a viral component that are essential to the viral life cycle.

Currently many antiviral therapies are available still in some cases, response rates and efficiency were lower. Higher doses and prolonged therapy leading to an increased side-effect. Also, the cost effective of the antivirals is another reason for alternative treatments. Most of the people are not affordable to the transplantation procedures. Therefore innovative, more valuable and less toxic agents are vital for HCV treatment. Some medicinal plant extracts such as *Glycyrrhiza glabra, Solanum nigrum* and *Phyllanthus amarus* are investigated in-vitro for the antiviral activity against HCV.

**Hepatitis C virus**

HCV is a single stranded positive sense RNA virus belongs to the family *Flaviviridae*. The whole viral genome of HCV is 9.6 kb nucleotide long encode a large polyprotein cleaved by viral and cellular proteases into certain structural and non-structural proteins (NS). Structural proteins include core protein and two envelope proteins (E1 and E2) are proteolytically slashed from the N-terminal segment of the viral polyprotein by cellular peptidases. Non-structural proteins include p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B are formed at the NS2-NS3 intersection by the NS2-NS3 metalloprotease and at the downstream position of NS3 by the NS3serine protease [De Francesco R *et al*., 2000].



HCV is grouped into seven major genotypes and 84 subtypes respectively [Smith DB *et al.,* 2014]. Among these 7 genotypes, the predominance of genotype 3 is seen in the northern, western, and eastern regions of India, whereas genotype 1 is most commonly found in southern region of India. Phylogenetic analysis of HCV strains isolated in various regions of the world have been identified. The genotype influences the disease course and the response to antiviral therapy [Simmonds *et al*., 2013]. Genotype 3 is the most profoundly distributed HCV strain globally, precisely accounting its contribution in the wide regions of Europe, North America and Australia. The genomic RNA contains one single open reading frame (ORF) which encoding a polyprotein that can be processed into 10 viral proteins after translation [Guoli Shi *et al*., 2018].

**Transmission of HCV**

HCV is a blood borne virus. Mode of transmission of infection occurs especially via exposure to contagious blood those constituting transfusion of unscreened contagious blood. Reusing of injections among drug users, Usage of unsterile medical and dental equipment’s. Transmission of infection from mother to foetus via perinatal route injections and rare estimates of sexual transmission and approximately 6% of infection is transmitted from mother to infants [Lauer GM *et al*., 2001]. It primarily infects the hepatocytes and evades innate and adaptive immunity. More than 50% of people infected with the HCV will transits chronic infection and 5-25% of people with chronic infection will develop liver cirrhosis over 10-20 years.

**Pathogenesis of HCV**

The viral particle is about 50-80nm in diameter and eventually associated with neutral lipids (cholesterol, ester and triglycerides) and apolipoproteins. In initial phase, cell attachment takes place during the interactions of apolipoprotein E with cell surface heparin sulphate proteoglycans [Denolly, S *et al*., 2018]. The envelope protein plays a major role in viral entry and in virus tropism to hepatocytes, cause acute hepatitis C, following acute infection, 50-80% of patients develop chronic hepatitis C which might lead to liver fibrosis, cirrhosis, hepatocellular carcinoma and death.

**Attachment**

Attachment of HCV on host cell receptor is achieved by E1 and E2 enveloped glycoproteins, further binding is mediated by apolipoproteins at the lipo surface and cell surface molecules. Enveloped proteins E1 and E2 interacts with CD81 and class B member 1 receptor, claudin 1, occuludin, claudin 6 or claudin 9, epidermal growth factor plays crucial role in viral entry [Zeisel,felmlee *et al*., 2013]. These complexes facilitate uptake and ensures species specificity.

**Penetration and uncoating**

Post viral attachment virus enters into the host cell mediated by clathrin endocytosis virus fuses with host endosomal membranes which progresses with liberation of viral nucleocapsid in the cytoplasm post uncoating of viral nucleocapsid is followed by release of positive stranded RNA into cytosol which acts as mRNA for synthesis of viral polyprotein.

**Protein Synthesis**

 HCV open reading frame translating region is controlled by internal ribosome entry site positioned in 5’ Un-translated region at the endoplasmic reticulum where polyproteins are translated into series of proteins 3 structural proteins and 7 nonstructural proteins. Host cellular peptidase plays crucial role in processing of HCV structural proteins, Viral peptidases are associated in processing of HCV non-structural proteins. After processing the viral proteins was found to be associated with intracellular membranes [Moradpour and Penin *et al.*, 2013].

**Replication**

 NS5B replicase protein catalyzes for viral replication. For RNA binding NS5A serves as dimer with channel for binding. Domain of NS5A protein is necessary for RNA replication complex, NS3 helicase unwinds the RNA strands and displaces the RNA binding proteins. Regulation of viral replication is controlled by helicase NS5 domain of NS3 and NS5A. NS4B forms a replication complex or ‘membranous web’ thus facilitating in HCV replication. HCV contains positive sensed RNA which serves as template for the synthesis of complementary negative strand which in turn acts as template to produce numerous positive strand genomes which can used for translating polyprotein which subsequently packed into new viral particle or new intermediates were produced [Seigel, Bengsch *et al.*, 2013].

**Assembly and Release**

Core and NS5A protein interact with RNA in cytoplasm which results in initiation process of viral particle formation. For the assembly and release of Virus, HCV uses VLDL production pathway. After assembly of viral components maturation of virus takes place, transfer of nascent particles across the ER membrane to enable access to the secretory pathway in hepatocytes. LDs and VLDL assembly pathway occur in ER lumen. There are three categories in HCV virion production initial phase where LDs assemble. VLDL assembly pathway engage to facilitate virion maturation.

**Treatment**

In 2014, Ledipasvir-Sofosbuvir where Ledipasvir is NS5A inhibitor and Sofosbuvir is NS5B inhibitor was approved by FDA is used to treat HCV genotype. In 2016, FDA approved combination of drugs Elbasvir-grazoprevir where Elbasvir is NS5A inhibitor and grazoprevir is NS4/3 inhibitor and Sobosbuvir-Velpatasvir, it can be used in combination with ribavirin in people with moderate to severe cirrhosis. Epclusa was the first medication to treat all six HCV genotypes. It provides a much-needed choice for patients with HCV genotype 3 infections, including those with compensated cirrhosis and its level significantly reduced with acid-reducing agents particularly proton-pump inhibitors.

Sofosbuvir-Velpatasvir-Voxilaprevir (vosevi) and Glecaprevir-Pibrentasvir (Mavyret) approved in 2017 is used to treat any HCV genotype. It is first treatment that can be administrated for only 8 weeks in people without cirrhosis. Most of the other combination drugs must be administered for a minimum of 12 weeks. Currently many antiviral therapies are available still in some cases, response rates and efficiency were lower. Higher doses and prolonged therapy leading to an increased side-effect. Also, the cost effective of the antivirals is another reason for alternative treatments. Most of the people are not affordable to the transplantation procedures. Therefore innovative, more valuable and less toxic agents are vital for HCV treatment. Some medicinal plant extracts such as *Glycyrrhiza glabra, Solanum nigrum* and *Phyllanthus amarus* are investigated in-vitro for the antiviral activity against HCV.

**Medicinal plants**

***Glycyrrhiza glabra***

*Glycyrrhiza glabra* belongs to Leguminosae family, kingdom: Plantae. Its binomial name is *Glycyrrhiza glabra Linn*. It is herbaceous perennial, height about 1m with pinnate leaves about 7-15cm long, with 9-17 leaflets. The flowers are purple to pale whitish blue, produce in loose inflorescence. The *Glycyrrhiza* shrub is a member of the family and grows in subtropical climates in rich soil [Siracusa *et al*., [2011](https://onlinelibrary.wiley.com/doi/full/10.1002/ptr.6178#ptr6178-bib-0115)]

The roots of *Glycyrrhiza glabra* contains a several active compounds including flavonoids, such as liquirtin, rhamnoloquirilin, liquiritigenin, prenyllicoflavone A, glucoliquiritin apioside, 1-metho-xyphaseolin, shinpterocarpin, shinflavanone, licopyranocoumarin, glisoflavone, licoaryl coumarin and coumarin- GU-12, and saponins, glycyrrhizin. Glycyrrhizin is a saponin compound as well as its aglycone glycyrrhetinic acid, are the potent components in *Glycyrrhiza glabra. Glycyrrhiza glabra* has various type of activities includes anti-tissue, anticoagulant and memory enhancing activity, anti-carcinogenic and anti-mutagenic activity, antioxidant and anti-inflammatory activity, anti-diabetic and hepatoprotective activity.

***Phyllanthus amarus***

*Phyllanthus* is the genus of flowering plant in the family *Phyllanthaceae;* It is the largest genus in *Phyllanthaceae* family it is prevalent worldwide especially commonly found in tropical and subtropical Countries. *Phyllanthus amarus* is an annual herb which grows up to 60-70 cm in length, stems are quite often branch from angular or base root. Leaves are abundant, subsessile, distichous, stipulate, paripinnate with small leaflet. It has been successive treatment nomination in many liver abnormalities and infectious conditions counting liver failure, liver damage.

It is still being used from the past to treat stomach, genitourinary, kidney, liver and spleen related symptoms and abnormalities and has antagonistic properties such as antiviral, anti-bacterial, anti-parasitic, anti-microbial, anti-inflammatory, anti-cancer, anti-oxidant [Patel *et al*., 2007]. Its other antagonistic function hostile to liver disorders for instance hepatocellular carcinoma jaundice and other symptoms like flu, cold, kidney and gall bladder stones, serious infection namely tuberculosis and various viral infections.

It is gaining attention in scientific research fields for recent rediscovery of *Phyllanthus amarus* ability to implicate as a potent novel antiviral therapy against Hepatitis B, Hepatitis C and it has been screened in research trials as well. Extracted Phyto-compounds efficacy against the Hepatitis C infection and the secondary metabolic Phytocompounds present in the *Phyllanthus amarus* are diverse classes with therapeutic benefits including flavonoids, alkaloids, tannins (Ellagitannins), polyphenols, major lignans, triterpenes, sterols and volatile oil. In leaves, the concentration of phyllanthin and hypophyllanthin has been found to be high, in contrast with stem were present in lower quantities.

***Solanum nigrum***

*Solanum nigrum* is a medicinal plant belonging to the family *Solanaceae*. *Solanum nigrum* is characterized by its white flowers and purple-black berries. *Solanum nigrum* showed anti-cancer activity for hepatocellular carcinoma cells [Wang C.W. *et al*., 2015]. The extracts of the *Solanum nigrum* contain many polyphenolic compounds. The leaves are rich in polyphenols, including phenolic acids and flavones.

*Solanum nigrum* is found to have anti-viral, anti-bacterial activity, anti-fungal activity, anti-diabetic activity, immune-stimulant activity, antioxidant activity, anti-HCV activity, cardio-protective activity, analgesic activity, anti-inflammatory activity, anti-diarrheal activity, cytotoxic activity, larvicidal activity and anti-seizure activity.

Mainly *Solanum nigrum* has been extensively used traditionally to treat various ailments such as pain, inflammation, and fever. Plant is also used in the oriental systems of medicine for various purposes as an anti-tumorigenic, antioxidant, anti-inflammatory, hepato-protective diuretic and antipyretic agent. Various compounds have been identified which are responsible for diverse activities.

Phytochemical investigation of the whole plant reported contains, alkaloids, flavonoids, tannins, saponins, glycosides, proteins, carbohydrates, coumarins and phytosterols. It has been found that *Solanum nigrum* contains the substances such as total alkaloids, steroid alkaloid, steroidal saponins and glycoproteins exhibiting antitumor activity [Zakaria ZA *et al.,* 2006]

**Conclusion**

HCV infection so far, a global threatening till now. In the era of vaccines, we don’t have a perfect suitable vaccine for HCV infection. Instead, there is lot of drugs available for the treatment of HCV infection, but there is lot of cases, HCV doesn’t completely eradicate while the progressive liver disease once fibrosis was formed. Actually, DAAs may not be effective, once chronic HCV infection headway to the stages of fibrosis, cirrhosis and HCC. Particularly, these drugs have so many drawbacks which may leads to finding of an alternative drug for treating HCV infections. So, the alternative medicine from the medicinal plants would be leads a good resource.

Herbal medicine has been used for centuries to treat a variety of ailments, including viral diseases, and has become a focal point for identifying, isolating, and purifying new entities to treat diseases such as HCV. According to estimates, 25% of commonly used medicines contain compounds isolated from plants. Van Rossum *et al.,* 1998 had demonstrated that Glycyrrhizin and glycyrrhetinic acid from *G. glabra* have been shown to protect against drug-induced liver injury, and long-term use of glycyrrhizin has been shown to protect against the development of hepatocellular carcinoma in chronic hepatitis. *P. amarus* treatment restores ethanol-induced liver injury by restoring normal levels of aspartate transaminase (AST), alanine transaminase (ALT), high-sensitivity human thyroglobulin (HTG), and tumour necrosis factor (TNF) according to Pramyothin P *et al.,* [2007]. Singh DP *et al.,* 2015 was tested *S. nigrum* for its ability to protect the liver from paracetamol-induced hepatotoxicity.

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