

MEDICINAL PLANTS AS ALTERNATIVE ANTI-HCV DRUGS

Abstract

Hepatitis C virus (HCV) is a serious public health concern that affects millions of people worldwide, causing liver cirrhosis and hepatocellular carcinoma (HCC). While there is no effective anti-HCV vaccine, the availability of anti-HCV drugs has streamlined the management of viral hepatitis and its complications. There are currently many DAA's available to help keep HCV infections under control. Strong doses and long-term treatment evoking an increased side-effect in addition to being expensive. The facts necessitate the development of newer anti-HCV drugs, which are currently being pursued. Looking for alternative drugs for natural resources is one approach to discovering novel anti- HCV drugs. In this study, we evaluated the anti-HCV properties of extract of *G. glabra*, *S. nigrum* and *P. amarus*.

Keywords: Medicinal Plants, Alternative Anti-HCV Drugs, Hepatocellular Carcinoma (HCC), Hepatitis.

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I. INTRODUCTION

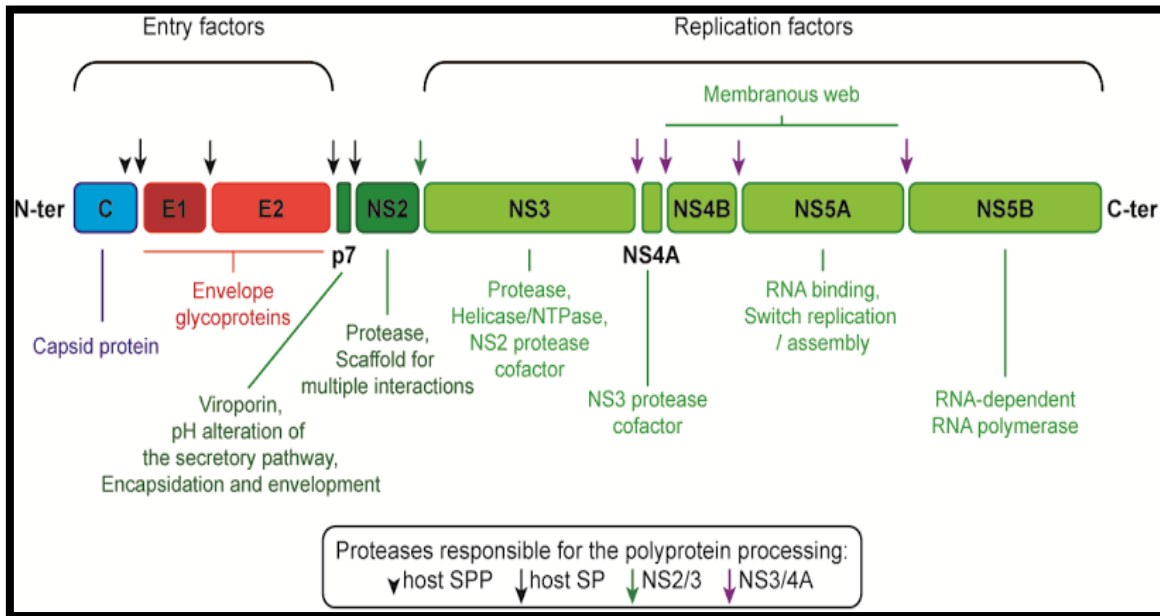
Hepatitis is a chronic inflammatory liver disease. The liver is an essential organ that process nutrients, filters the blood, and fights against infections. When the liver is inflamed or damaged, its function can be affected, due to the variety of non-infectious agents like massive alcoholic, toxins, certain medications and medical conditions. Despite that infectious virus usually causes hepatitis. Hepatitis virus has five strains as types A, B, C, D, and E. All of them produce liver disease, while vary in modes of transmission, severity of the infection, geographic distribution and prevention methods. Hepatitis may range from acute and chronic infection. Particularly, types B and C leads to chronic condition in hundred million of individuals and the main cause of hepatic cirrhosis, hepato-cellular carcinoma and viral hepatitis-related deaths.

There are effective vaccines are available for Hepatitis A and B virus, while no vaccine for Hepatitis C infection. Most patients infected with HCV infection get rid of the virus themselves, but in most cases, the intense stage of hepatitis C infection may lead to chronic stage of infection. Since there is no vaccine available for HCV, antiviral therapy is suggested. In the late 2000s, FDA has approved combination antivirals such as a Ribavirin and pegylated Interferon- α which were effective initially, owing to drastic side effects that have been exhibited is an escalating global health issue. Such modern drugs contain direct-acting antiviral (DAA) agents, especially 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 certain circumstances, reaction rates and efficiency were decreases. Strong doses and long-term treatment evoking 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. Hence inventive, more desirable and less destructive agents are essential to treat HCV. Some medicinal plant extracts such as *Glycyrrhiza glabra*, *Solanum nigrum* and *Phyllanthus amarus* are studied in-vitro for the antiviral activity against HCV.

II. HEPATITIS C INFECTION

HCV belongs to the family Flaviviridae, possess single stranded positive sense RNA virus. The entire genome of HCV is about 9.6 kb nucleotide long encode a large polyprotein cleaved into certain structural and non-structural proteins (NS). Structural proteins include core protein and two envelope proteins (E1 and E2) which 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 prevalence of genotype 3 was seen in the northern, western, and eastern regions of India, although genotype 1 is most typically found in southern region of India. HCV strains were isolated from various regions of the world for phylogenetic analysis. The genotype influences the course of disease 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. After translation, around 10 viral proteins were processed from the polyprotein which encoded in open reading frame (ORF) of genomic RNA [Guoli Shi *et al.*, 2018].

1. **Transmission of Hepatitis C Virus:** Hepatitis C infection is transmitted from a blood borne virus. Mode of transmission of infection occurs especially via exposure to contagious blood those constituting transfusion of un-screened 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 transit chronic infection. More than 5-25% of individuals with chronic infection will produce hepatic cirrhosis over 10-20 years.
2. **Pathogenesis of HCV:** The viral particle is about 50-80nm in diameter and eventually combined with neutral lipids and apo-lipoproteins. In early stage, cell attachment takes place when the interactions between apolipoprotein E and cell surface heparin sulphate proteoglycans [Denolly, S *et al.*, 2019]. The envelope protein plays a vital role in viral entry and tropism to hepatocytes, cause acute hepatitis C infection, around 50-80% of patients develop chronic hepatitis C which might lead to hepatic 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 interacting with CD81, class B member 1 receptor, claudin 1, occludin, 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 the HCV structural proteins, Viral peptidases are involved in processing the 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 sense RNA which acts as a template for the synthesis of complementary negative strand which in turn acts as template to produce numerous positive strand genomes which can be 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, nascent particles across the endoplasmic reticulum membrane to provide 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. The pathway of VLDL engaged to facilitate the maturation of virion.
3. **Treatment:** In 2014, Ledipasvir-Sofosbuvir where Ledipasvir is NS5A inhibitor and Sofosbuvir is NS5B inhibitor was authorized by FDA is used for HCV genotype. In 2016, FDA approved combination of drugs Elbasvir-grazoprevir where Elbasvir is NS5A inhibitor and grazoprevir is NS4/3 inhibitor and sofosbuvir-Velpatasvir, can be used as a combination with ribavirin in individuals with moderate to severe cirrhosis. The first medication to treat all genotypes of HCV was Epclusa. It allows a required choice for

patients with HCV genotype 3 infections, including those with compensated cirrhosis and its level greatly reduced with acid-reducing agents particularly proton-pump inhibitors.

In 2017, Sofosbuvir-Velpatasvir-Voxilaprevir (vosevi) and Glecaprevir-Pibrentasvir (Mavyret) were approved to treat any HCV genotype. It is the first line treatment that can be administered in people without cirrhosis for only 8 weeks. In most other combined drugs must be administered for a minimum of 12 weeks. Currently many antiviral therapies are available still in certain cases, response rates and efficiency were reduced. High doses and long-term treatment lead 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. Hence novel, better and less destructive agents are vital for HCV treatment. Some medicinal plant extracts such as *Glycyrrhiza glabra*, *Solanum nigrum* and *Phyllanthus amarus* are studied in-vitro for the antiviral activity against HCV.

III. MEDICINAL PLANTS

1. ***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 leafed about 7-15cm long and 9-17 leaflets. The flowers are purple to pale whitish blue, produce in loose inflorescence [Siracusa *et al.*, 2011].

The roots of *Glycyrrhiza glabra* contains numerous active components including flavonoids, such as liquirtin, rhamnoliquiritin, liquiritigenin, prenyllicoflavone A, glucoliquiritin apioside, 1-methoxyxyphaseolin, shinpterocarpin, shinflavanone, licopyranocoumarin, glisoflavone, licoaryl coumarin and coumarin- GU-12, and saponins, glycyrrhizin. Glycyrrhizin is a saponin compound and its aglycone glycyrrhetic 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.

2. ***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 abdomen, genitourinary, kidney, liver and spleen related symptoms, abnormalities and has antagonistic properties such as antiviral, anti-bacterial, anti-parasitic, anti-microbial, anti-inflammatory, anti-cancer, antioxidant [JR Patel *et al.*, 2011]. 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, polyphenols, major lignans, sterols, triterpenes 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

- 3. *Solanum Nigrum*:** *Solanum nigrum* is belonging to the family *Solanaceae*. It is characterized by its white flowers and purple-black berries. *Solanum nigrum* possess anti-cancer against hepatocellular carcinoma [Wang C.W. *et al.*, 2015]. The extracts of the *Solanum nigrum* include many polyphenolic compounds. Numerous components have been identified which are responsible for diverse activities includes anti-viral, anti-bacterial, anti-fungal, anti-diabetic, 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 widely used to treat various ailments such as pain, inflammation, and fever. Phytochemical investigation reports that whole plant 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]

IV. 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 to treat 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 Hepatitis C infection headway to the stages of hepatic fibrosis, hepatic 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 remedies have been used over the years to treat a type of illness, including viral diseases, and has come to be a focal point for identifying, isolating, and purifying new entities to treat diseases such as HCV. As estimates, 25% of used medicines commonly contain compounds isolated from plants. Van Rossum *et al.*, 1998 had demonstrated that Glycyrrhizin and glycyrrhetic acid from *G. glabra* have been shown to protect against drug-induced liver injury, and prolonged use of glycyrrhizin have shown to protect against the emergence of hepatocellular carcinoma from chronic hepatitis. *P. amarus* treatment restores ethanol-induced liver disease 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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