Review of Literature
The liver is the largest gland in the body. It is an extremely large and important organ. The liver takes up the space on the right-hand side that is occupied by the stomach on the left. It is firmly pushed up against the right dome of the diaphragm where the inferior vena cava, which passed through the liver, enters the thoracic cavity. It is held in place by folds of peritoneum. This peritoneal folding produces the falciform ligament anteriorly which attaches to the ventral abdominal wall and the coronary ligament superiorly which attaches to the diaphragm. The liver itself is divided into four lobes, with the left and right lobe being by far the largest, with the caudate and quadrate lobes being small lobes near the vena cava and the gall bladder respectively. Microscopically, the liver is organized into hepatic lobules. These consist of a small central vein with columns of hepatic cells radiating outwards from this central point. Blood flows readily inwards towards the central vein between the columns from both hepatic arteries and branches of the hepatic portal vein. The hepatic portal vein drains the portal venous system and contains high concentrations of digestion products that are processed by cells in the liver called hepatocytes. Also present in the lobules are hepatic macrophages that engulf most of the bacterial cells that have entered through the intestinal wall. These are fine tubes that drain the secretory product bile out to the periphery of the lobule where the tubes unite to form hepatic ducts which eventually unite to form the common hepatic duct that carries bile out of the liver.

The functions of the liver are varied, working closely with nearly every fundamental system and process in the human body. It produce substances that break down fats, convert glucose to glycogen, produce urea-the main substance of urine, make certain amino acids-the building blocks of proteins, filter harmful substances from the blood such as alcohol, stores vitamins and minerals like vitamins A, D, K and B12 and maintain a proper level of glucose in the blood. The liver is also responsible for producing cholesterol. It produces about 80% of the cholesterol in human body.
Liver disease is a broad term describing any number of diseases affecting the liver. Some of the diseases are hepatitis, cirrhosis, liver cancer and Wilson's disease—a chronic inflammation that progresses ultimately to organ failure. Alcohol alters the metabolism of the liver, which can have detrimental effects if alcohol is taken for a very long periods of time. Hemochromatosis also cause liver problems. Liver disease patients show many symptoms. The external signs include a coated tongue, bad breath, skin rashes, itchy skin, excessive sweating, offensive body odour, dark circles under the eyes, red swollen and itchy eyes, acne rosacea, brownish spots and blemishes on the skin and flushed facial appearance or excessive facial blood vessels. Other symptoms include jaundice, dark urine, pale stool, bone loss, easy bleeding, itching, small, spider-like blood vessels visible in the skin, enlarged spleen, fluid in the abdominal cavity, chills, pain from the biliary tract or pancreas and an enlarged gallbladder. The symptoms related to liver dysfunction include both physical signs and a variety of symptoms related to digestive problems, blood sugar problems, immune disorders, abnormal absorption of fats and other metabolic problems.

Liver diseases most likely to be seen in children include:

- galactosemia, an inherited disease in which the body can not tolerate certain sugars in milk. These sugars can build up, causing serious damage to the liver and other organs of the body.
- Alagille's syndrome, a condition in which the bile ducts narrow and deteriorate, especially during the first year of life
- alpha 1- antitrypsin deficiency, a genetic liver disease in children that can lead to hepatitis and cirrhosis of the liver
- neonatal hepatitis, that occurs in a newborn during the first few months of life
- tyrosinemia, a disorder that causes serious problems with liver metabolism
- hemorrhagic telangiectasia, a condition in which thin blood vessels allow frequent and easy bleeding of the skin and digestive tract
- Reye's syndrome, a condition that causes a buildup of fat in the liver. This condition has been linked in some cases to use of aspirin, especially in conjunction with chickenpox, influenza, or other illnesses with fever.
- Wilson's disease, an inherited condition that causes a buildup of the mineral copper in the liver
- thalassemia, a group of hereditary anemias, or low red blood cell counts
• biliary atresia, a condition in which the bile ducts extending from the liver to the intestine are too small in diameter or are missing.

Liver diseases in adults include:
• cirrhosis, which is a serious condition that causes tissues and cells in the liver to be replaced by scar tissue.
• type I glycogen storage disease, which causes problems in controlling blood sugars when a person fasts
• porphyria, a condition that causes a malfunction in how the body uses porphyrins.

Alcohol-related liver diseases include:
• fatty liver disease, which causes an enlarged liver
• alcoholic hepatitis
• alcoholic cirrhosis

Liver disease can be caused by a variety of factors. Causes include, viral or bacterial infections, congenital birth defects or abnormalities of the liver present at birth, metabolic disorders or defects in basic body processes, alcohol or poisoning by toxins, certain medications that are toxic to the liver, nutritional deficiencies and trauma or injury.

Hepatitis
The word hepatitis simply means an "inflammation of the liver" without pinpointing a specific cause. Hepatitis may be due to one of the following reasons. That is it may be due to
• viral or bacterial infection of the liver
• liver injury caused by a toxin (poison)
• or liver damage caused by interruption of the organ's normal blood supply
• or may be experiencing an attack by his or her own immune system through an autoimmune disorder
• or have experienced trauma to the abdomen in the area of the liver
Hepatitis may also result due to alcohol consumption, the typical histologic picture includes hepatocellular necrosis and ballooning degeneration, alcoholic Mallory's hyaline bodies and an inflammatory reaction with many polymorphonuclear leukocytes (sattelitosis). It is estimated that 15 - 20 years of excessive drinking is necessary to develop alcoholic hepatitis. Cholestasis is prominent. It is more severe in females and also in Northern Europeans. It is unrelated to pattern of drinking or type of alcohol drink. High mortality rates are seen (30 - 60%) and patients often deteriorate after hospital admission despite abstinence. Alcoholic hepatitis has been established as an important precursor to the formation of cirrhosis. The most common cause is viral infection that too by a group of viruses called hepatitis group of viruses which includes HAV, HBV, HCV, Hepatitis delta agent or HDV and HEV. All these viruses can cause an acute disease with symptoms lasting several weeks including yellowing of the skin and eyes (jaundice), dark urine, extreme fatigue, nausea, vomiting and abdominal pain. It can take several months to year to feel fit again. Among the hepatitis group of viruses HAV and HEV are water borne where as HBV, HCV and hepatitis delta agents are blood borne viruses. Some hepatitis viruses can mutate, which means they can change over time and can be difficult for the body to fight. The patient then will need a liver transplant to survive, which is not always available or successful. In some rare cases, the Epstein Bar Virus can also result in hepatitis because it can cause inflammation of the liver. Other viruses like varicella and cytomegalovirus (CMV) can also cause hepatitis. Helicobacter pylori, a bacterium can also affect liver and cause hepatitis.

HAV

HAV, formerly known as infectious hepatitis is an acute infectious disease of the liver caused by Hepatitis A virus (HAV). HAV is a major global public health problem especially in developing countries (Hollinger et al., 1999). HAV causes an acute infection of the liver. The infection is usually self limiting but typically produces fever, malaise, anorexia, nausea and abdominal discomfort. The severity of disease and mortality increases in older age groups. Complications of HAV infection can include fulminant hepatitis, cholestatic hepatitis and relapsing hepatitis (WHO, 2000). HAV is classified within the genus Hepatovirus of the family Picornaviridae. This classification is based on several unique features of HAV: liver cell tropism, small and possibly absent VP4 protein, a unique VP1-2A precursor protein (pX), striking
thermostability, a relatively slow and usually noncytopathic replication cycle and a
strong tendency to initiate persistent infections in cell culture.

The virion of HAV is approximately 27 nm in diameter and appears roughly
spherical by electron microscopy. Based on the known structure of other
picornaviruses, the HAV capsid is thought to have icosahedral symmetry and contains
60 copies of each of the three major polypeptides: 1B (VP2), 1C (VP3) and 1D (VP1).
HAV genome is a single-stranded, positive-sense RNA, approximately 7500 bases in
length. As in other members of the picornavirus family, a short genome-linked protein
is covalently linked to the 5’ end of the RNA, while at the 3’ end is a poly (A) tract.
Flanking the single large ORF are 5’ and 3’ nontranslated regions of approximately 735
and 64 bases, respectively.

With regard to the pathogenesis of HAV there are no known differences in the
pathogenicity of different HAV strains, although few studies have examined this in
detail. The currently available evidences suggest that HAV is not directly cytopathic for
the hepatocytes, but rather induces diseases through an immunopathologic mechanism.
The most important factor influencing disease severity associated with HAV infection
is patients age at the time of infection. Very young children, under the age of 2 years,
seldom develop symptoms of classic viral hepatitis (Sheila Sherlock and James Dooley,
2002). In contrast, the seroprevalence in several industrialized countries in pediatric
ages are low and infection is usually acquired during late adolescence and early
adulthood which is accompanied by significant morbidity (WHO, 2000). Their
infections, although possibly not asymptomatic, are seldom icteric. The reverse may be
said for adults. HAV causes only acute hepatitis and has never been unequivocally
associated with chronic disease or persistent infection. Infections in older children and
adults are usually symptomatic, with the majority exhibiting jaundice (Alborzi et al.,
2003). Complications increase significantly in adults with mortality rate of up to 2.1%
in patients more than 40 years. Prevalence of HAV in developing countries is very high
(98%) (Xavier and Anish, 2003). Patients with negative titres were generally younger.
An earlier study from Shiraz city (Alborzi et al., 2003) revealed a high rate of
immunity in children (68%) among 15 year of age and seropositive children had higher
mean number of house hold members compared to the seronegative ones. The rate in
Tehran among school children was 22.3% (Mehr et al., 2004) and 92.3% in a report from Delhi (Acharya et al., 2003).

HAV is shed in the feces of acutely infected individuals and transmission of the infection is almost always by the fecal-oral route. Virus shed in the feces is largely replicated within hepatocytes in the liver and gains access to the intestinal contents by passage through the biliary tract. HAV is found in most human populations. However, the virus enjoys generally wider circulation in developing countries with relatively poor public sanitation standards. HAV rates increased significantly among illicit users of injected drugs in the United States during the late 1980s. Increased risk for HAV transmission exist within closed institutions, such as prisons and institutions (Franco, 2003). Worldwide, it is estimated that about 1.5 million clinical cases of HAV occur each year (Lavanchy, 2002). Multitransfused adult beta-thalassemic patients present higher frequency of anti-HAV IgG antibodies than normal population of the same geographic area. This difference is difficult to explain, but it can be attributed to the higher vulnerability of thalassemics to HAV infection and to passive transfer of anti-HAV antibodies by blood transfusions (Siagris et al., 2008). Analysis of 1612 subjects in different parts of India demonstrated that almost 50% of children under 5 years of age are at risk for HAV (Mall et al., 2001). A recent report showed that the relative contribution of HAV to acute viral hepatitis in children has increased to over 80% in 1994 - 1997 as compared to 51% in 1978 - 81 (Chadha et al., 2003). However, a number of studies in school children in northern and southern India have reported evidence of prior infection in up to 98% of 10-year-old children (Batra et al., 2002, Mohanavalli et al., 2003). The recent studies have shown a decline in anti-HAV seroprevalence in Latin America, which has generally been explained by improvements in sanitary conditions particularly in the access to clear water and to sewage systems (Jacobsen and Koopman, 2004, Tanaka, 2000 and Tapia-Conyer et al., 1999) and by improvements in living standards (Jacobsen and Koopman, 2004). Patients with acute HAV usually require only supportive care, with no restrictions in diet or activity. Hospitalization might be necessary for patients who become dehydrated because of nausea and vomiting and is critical for patients with signs or symptoms of acute liver failure. Medications that might cause liver damage or are metabolized by the liver should be used with caution among persons with HAV. There is no specific treatment for hepatitis A. Rest is recommended during the acute phase of the disease when the
symptoms are most severe. People with acute hepatitis should avoid alcohol and any substances that are toxic to the liver.

Active immunization against HAV can be achieved with inactivated HAV vaccines produced by several commercial manufactures. These vaccines consist of HAV that has been propagated in cell culture, purified and inactivated with formalin. A review of cost-effectiveness of HAV vaccine in developed countries concluded that vaccination is likely to be cost effective in institutions (Rosenthal, 2003). Study conducted in England and Wales state that HAV vaccination should be considered for those individuals with special needs whose capacity to maintain good standards of hygiene is limited following a risk assessment (Crowcroft et al., 2001). The epidemiology of HAV has changed fundamentally with the advent of HAV vaccine (CDC, 2006). Before that it was primarily cyclical but this is now changing in the US (CDC, 2006), the UK and Scandinavia (Hawker et al., 2005). Neither the World Health Organization nor the Center for Disease Control and Prevention in Atlanta (USA) lists people in institutions for intellectual disabilities as a high-risk group needing HAV vaccine (WHO, 2000). However, this client group is vaccinated in a number of European countries according to the findings of the EUROHEP.NET project (Leuridan et al., 2005). As almost all hepatitis A infections are transmitted by the fecal-oral route, good sanitation practices, including high standards of quality for public water supplies, have resulted in low prevalence of HAV infections in many well-developed societies.

**HBV**

Infection by HBV is a worldwide public health problem. It is a significant cause of morbidity and mortality, especially in developing countries. HBV is a member of the *Hepadnavirus* family. The virus particle, (virion) consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity. The outer envelope contains embedded proteins which are involved in viral binding and entry into susceptible cells. The virus is one of the smallest enveloped animal viruses with a virion diameter of 42 nm, but pleomorphic forms exist, including filamentous and spherical bodies lacking a core. These particles are not infectious and are composed of the lipid and protein that forms part of the surface of the virion, which is called the surface antigen (HBsAg), and is produced in excess during the life cycle of the virus.
The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded. One end of the full length strand is linked to the viral DNA polymerase. The genome is 3020-3320 nucleotides long (for the full length strand) and 1700 - 2800 nucleotides long (for the short length strand). HBV infection is the most common cause of chronic hepatitis, liver cirrhosis and (Hepato Cellular Carcinoma) HCC worldwide (Kane, 1998).

There are six genotypes (A-F) of HBV which have been identified. However, the genotype-related differences in the pathogenicity of HBV remain unknown. Based on an intergroup divergence of 8% or more of the complete genomes, HBV can be classified in to 7 genotypes, i.e. A-G (Stuyver et al., 2000, Naumann et al., 1993 and Norder et al., 1992). Genotype H was recently identified in central America (Arauz-Ruiz et al., 2002). It is well known that HBV genotypes have distinct geographical distributions. The prevalent HBV strains in China are genotype B and C (Zhu et al., 1999), but the two genotypes distribute unevenly in China. In northern China, genotype C is predominant (85.1%), while in southern China, genotype B is predominant (55.0%). Genotypes A and D are also found in other areas of China. However, the genotypes E-H have not been reported in China. Recently, genotype C/D hybrid was identified in Tibet (Cui et al., 2002) and genotype B was found recombinated with preC/C region of genotype C in China (Luo et al., 2004). Accumulated data suggest the importance of genotype, subgroup and recombination may influence the biological characteristics of virus and clinical outcome of HBV infection. Several studies reported a correlation of HBV genotypes with HBeAg clearance, liver damage and the response to IFN treatment. HBV carriers with genotype B have lower histologic activity scores (Lindh et al., 1999) and genotype C is more prevalent in patients with cirrhosis (Ding et al., 2001 & Kao et al., 2000). Furthermore, a retrospective study showed that HBV genotype B is associated with a higher rate of IFN-induced HBeAg clearance compared with genotype C (Kao et al., 2000). The response of different HBV genotypes to interferon-alfa treatment is of increasing interest because the benefit of interferon-alfa or its pegylated form in combination with other antiviral agents is being explored in the treatment of chronic HBV. In a homogeneous group of prospectively followed patients from Europe a recent study demonstrates that genotype A responds better than other HBV genotypes to standard interferon therapy and represents an independent predictor
of a therapeutic success, with a greater impact than other pre-treatment characteristics, such as HBV DNA or ALT levels.

HBV primarily interferes with the functions of the liver by replicating in liver cells, known as hepatocytes. During HBV infection, the host immune response causes both hepatocellular damage and viral clearance. Although the innate immune response does not play a significant role in these processes, the adaptive immune response, particularly virus-specific Cytotoxic T Lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. By killing infected cells and by producing antiviral cytokines from viable hepatocytes, CTLs eliminate the virus. Virological factors in the pathogenesis of HCC have recently been defined. Both retrospective and prospective studies strongly supported the relation between positive HBeAg and the risk of HCC (Yang et al., 2002, Tsai et al., 1996 and Lin et al., 1991). A prospective study in Taiwan (Yang et al., 2002) showed that relative risk of HCC among men who were positive for both HBsAg and HBeAg were much higher than that among men who were positive for HBsAg alone. HBV DNA was identified as the most important predictor of the development of HCC in HBsAg positive patients with different clinical conditions (Ohata et al., 2004, Ikeda et al., 2003 and Ishikawa et al., 2003). Therefore, efforts at eradicating or reducing the viral load may reduce the risk for HCC. Additionally, HBV genotype might play a role in the development of HCC. The data from Taiwan showed that genotype C is associated with more severe liver disease including cirrhosis and HCC, whereas genotype B is associated with the development of HCC in young noncirrhotic patients.

HBV spread through contact with infected body fluids and the only natural host is human. Blood is the most important vehicle for transmission, but other body fluids have also been implicated including semen and saliva (Bancroft et al., 1997 & Scott et al., 1980). Currently, three modes of HBV transmission have been recognized: perinatal, sexual and parenteral/percutaneous transmission. There is no reliable evidence that airborne infections occur and feces are not a source of infection. HBV is not transmitted by contaminated food or water, insects or other vectors. Sexual transmission of HBV is a major source of infection in all areas of the world, especially in the low endemic areas, such as North America. HBV is considered to be a sexually transmitted disease (STD). For a long time, homosexual men have been considered to
be at the highest risk of infection due to sexual contact i.e. 70% of homosexual men were infected after 5 years of sexual activity (Alter, 2003).

Studies on the prevalence of HBV shows that 350 million people worldwide are chronic HBV carriers, representing approximately 7% of the total population. Among them 78% are in Asia, 16% in Africa, 3% in South America and 3% in Europe, North America and Oceania combined. Epidemiologic studies have demonstrated that there is a consistent and specific causal association between HBV infection and HCC. In patients with persistent HBV infection, the risk of HCC was 100 times higher than in non-infected individuals. The global distribution of HCC correlates with the geographic prevalence of chronic carriers of HBV. The highest rates are in Southeast Asia and sub-Saharan Africa, with the HCC incidence more than 50% of the total population (Bosch et al., 1999). The prevalence of HBV infection varies markedly throughout regions of the world (Kane et al., 1999). It was estimated that approximately 2 billion people have serological evidence of past or present HBV infection. More than 350 million are chronic carriers of HBV (WHO Fact sheet, 2000). It was reported that 15 - 40% of HBV infected patients would develop cirrhosis, liver failure or HCC (Lok, 2002).

Serological markers for HBV infection are numerous and the diagnosis and assessment of HBV infection are complex. In addition to the traditional markers of HBeAg and HBsAg (Pawlotsky et al., 2000, Pawlotsky et al., 1997, Kurstak et al., 1996 and Yang and Vyas 1996) the clinical utility of new serologic markers is being explored (Kuttner et al., 1999 & Kurstak et al., 1996). The serologic diagnosis of HBV infection is established by detecting either antibodies and/or their respective antigens i.e. the HBsAg and anti-HBs, HBcAg and anti-HBe (Zaaijer et al., 1994 & Hoofnagle et al., 1987). Enzyme immunoassay is the most commonly used test for HBsAg, whereas the most commonly used tests for anti-HBs employ both EIA and RIA methods (Kurstak et al., 1996). In a study conducted by Brazil revealed that the seroprevalence of anti-HBc increased with age, but decreased with education level in both genders (Nascimento et al., 2008). Tests for HBcAg are generally limited for use in testing liver biopsy samples. The presence of HBcAg in liver tissue indicates ongoing viral replication and is often interpreted as a measure of infectivity. Positive anti-HBc IgM indicate acute HBV infection (Kurstak et al., 1996). HBe antigen is a viral protein secreted by HBV infected cells. Its presence indicates high levels of virus
in the blood and it is an indicator of the infectiousness of the carrier. It was reported that HBeAg carrier status tends to be longer and the prevalence of HBeAg appears higher in patients with genotype C than with genotype B (Chu et al., 2002 & Orito et al., 2001). HBV DNA is assayed by polymerase chain reaction (PCR) methods, branched chain DNA (bDNA) methods and by nucleic acid hybridization methods. PCR based assays tend to be more sensitive, but bDNA and hybridization based assays may provide more specificity (Pawlotsky et al., 2000 & Roudot-Thoraval et al., 1997).

The main strategies available for the prevention of HBV infection are

1. Behavior modification to prevent disease transmission
2. Passive immunoprophylaxis and
3. Active immunization.

Acute hepatitis B infection does not usually require treatment because most adults clear the infection spontaneously. Treatment of chronic infection may be necessary to reduce the risk of cirrhosis and liver cancer. Although none of the available drugs can clear the infection, they can stop the virus from replicating, and prevent liver damage such as cirrhosis and liver cancer. Treatments include antiviral drugs such as lamivudine, adefovir, entecavir, and immune system modulators such as interferon alpha. However, some individuals are much more likely to respond than others and this might be because of the genotype of the infecting virus or the patient's heredity. The treatment works by reducing the viral load which in turn reduces viral replication in the liver.

Thyagarajan and his co-workers reported that a common plant extract from *Phyllanthus amarus* cleared the chronic carrier state of HBV (Thyagarajan et al., 1988). For determining the mechanism of action of *P. amarus*, HepG2 2.2.15 cells were used, which support HBV replication. *P. amarus* inhibited HBV polymerase activity, decreased episomal HBV DNA content and suppressed virus release into culture medium. To examine transcriptional control mechanisms, G26 hepatitis B virus transgenic mice was used, which produce serum HBsAg but neither HBcAg nor virion particles. When *P. amarus* was administered to transgenic mice, hepatic HBsAg mRNA levels decreased, indicating transcriptional or post-transcriptional down-regulation of the transgene. Increase in HBV mRNA expression after stimulation of the
glucocorticoid responsive element was also suppressed by *P. amarus*, suggesting involvement of the HBV enhancer in this response (Lee *et al.*, 2003). The whole plant extract of *P. niruri* have shown that there exist antiviral properties against HBV (Thyagarajan *et al.*, 1982 & Thyagarajan, 1979). *Phyllanthus amarus* possibly interrupts the interaction between HBV enhancer I and cellular transcription factors (Ott *et al.*, 1997). The aqueous extract of the dried whole plant did not produce any chronic toxicity in mice at 0.2 mg daily dose per animal for 90 days, as determined by physiological, biochemical and histopathological parameters (Jayaram *et al.*, 1987). In a clinical trial on chronic HBV carriers, HBsAg clearance in the *P. amarus* treated group was 59%, versus 4% in the placebo group (Thyagarajan *et al.*, 1988). The second open trail in 1990 showed 20% HBsAg clearance and 63.6% loss of infectivity by HBeAg sero-conservation (Thyagarajan *et al.*, 1990). Jayaram *et al.*, studying the effect of *P. amarus* on β-galactosamine-induced hepatotoxicity on isolated rat hepatocytes, found that *P. amarus* by itself was not hepatotoxic and at 1 mg/mL concentration it was found to be hepatoprotective (Jayaram *et al.*, 1994).

Immunization with HBV vaccine is the most effective means of preventing HBV related consequences. Preventing HBV transmission during early childhood is important because of the substantial likelihood of chronic HBV infection. The current overall risk of acquiring HBV after a transfusion is about one in 50,000 per recipient. Unfortunately donors who are in the early incubation period of their disease capable of transmitting HBV will remain unidentified with current therapies. In order to avoid unnecessary risk of HBV infection, patients who depend on recurrent transfusion should be vaccinated.

**Hepatitis Delta agent**

In 1977, gastroenterologist Mario Rizzetto of Turin, Italy reported the detection of a new hepatitis B specific antigen in some patients with chronic B hepatitis. The antigen termed ‘delta antigen’ was localized in the nuclei of hepatocytes, closely resembling the hepatitis B core antigen (HBCAg) in its subcellular localization. Its presence was always associated with HBV infection and patients with delta antigen also develop antidelta antibodies (Purcell and Gerin, 1996, Lai, 1994 and Rizzetto, 1977).
HDV is a single stranded RNA satellite virus, which depends on HBV as a helper virus for a complete viral life cycle. To date, HDV is the only member virus of the floating genus *Deltavirus* (no Family designation). No sequence or structural relationship exists between HDV and HBV. Structural and biochemical features of HDV RNA suggest some similarity to plant viroids or virusoids. However, *Deltavirus* is taxonomically distinct from viroids. HDV has been isolated only from humans. HDV isolates can be classified into three genotypes based on the extent of nucleotide sequence divergence, each genotype differing by over 35%.

The genome of HDV is unrelated to the genome of *hepadnaviruses* of which HBV is a member. HDV is therefore not a defective-interfering particle of HBV and should be considered as a satellite virus a natural subviral satellite of HBV (Hadziyannis, 1997, Lai, 1994 and Taylor, 1996). The genome of HDV was cloned and sequenced in 1986 (Wang, 1986). The envelop proteins on the outer surface of HDV are entirely provided by HBV (Taylor, 1996, Monjardino and Saldanha, 1990 and Makino, 1987). A significant difference between viroids and HDV is that viroids produce no proteins where as HDV produces two proteins called the small and large delta antigens (HDAg-S and HDAg-L, respectively). These two proteins are produced from a single open reading frame. They are identical for 195 amino acids and differ only by the presence of an additional 19 amino acids at the C-terminus of HDAg-L. Despite having 90% identical sequences, these two proteins play diverging roles during the course of an infection. HDAg-S is produced in the early stages of an infection and is required for viral replication. HDAg-L, in contrast, is produced during the later stages of an infection which acts as an inhibitor of viral replication and is required for assembly of viral particles. There is no evidence that the HBV-derived envelop proteins are additionally modified when they become envelop of HDV (Purcell and Gerin, 1996). Experimentally it can be transmitted to chimpanzees and woodchucks in the presence of HBV or woodchuck hepatitis virus (WHV) respectively (Negro, 1996, Purcell and Gerin, 1996, Taylor, 1996, Lai, 1995, Monjardino and Saldanha, 1990, Sureau, 1989). In HDV the outcome of disease largely depends on whether the two viruses infect simultaneously (coinfection), or whether the newly HDV infected person is a chronically infected HBV carrier (super infection). Coinfection of HBV and HDV results in both acute type B and acute type D hepatitis. The incubation period depends on the HBV titer of the infecting inoculum. Depending on the relative titres of HBV
and HDV a single bout or two bouts of hepatitis may be seen. Coinfections of HBV and HDV are usually acute, self-limited infections. The chronic form of HDV is seen in less than 5% of HBV-HDV coinfect ed patient (Hadziyannis, 1997 & Purcell and Gerin, 1996). Superinfection of HBV and HDV causes a generally severe acute hepatitis with short incubation time that leads to chronic type D hepatitis in up to 80% of cases. Superinfection is associated with fulminant acute hepatitis and severe chronic active hepatitis often progressive to cirrhosis (Purcell and Gerin, 1996 & Lai, 1994).

Fulminant viral hepatitis is rare but still about 10 times more common in HDV than in other types of viral hepatitis. It is characterized by hepatic encephalopathy showing changes in personality, disturbances in sleep, confusion and difficulty concentrating, abnormal behavior, somnolence and coma. The mortality rate of fulminant HDV reaches 80%. Liver transplantation is indicated (Purcell and Gerin, 1996 & Lai, 1994). Progression to cirrhosis usually takes 5 - 10 years but it can appear 2 years after onset of infection. About 60 to 70 % of patients with chronic HDV develop cirrhosis. A high proportion of these patients die of hepatic failure (Purcell and Gerin, 1996).

Two possible mechanisms of HDV pathogenesis heave been proposed, first is the direct cytocidal effects of HDV and the second is an immune-mediated pathology. The role of cellular immunity in the pathogenesis of HDV is not clear. HDV is present worldwide and in all age groups (Purcell and Gerin, 1996 & Lai, 1994). Infection with both HBV and HDV is associated with more severe liver injury than HBV infection alone (Lai, 1995). Pathologic changes in HDV are limited to the liver, the only organ in which HDV has been shown to replicate. The histologic changes consist of hepatocellular necrosis and inflammation (Purcell and Gerin, 1996). HDV genome replication is not acutely cytopathic and both humoral and cellular immune mechanisms may be involved in the pathology of HDV. More experimental data are needed to unravel the underlying mechanisms of HDV induced disease (Hadziyannis, 1997, Purcell and Gerin, 1996, Taylor, 1996 and Lai, 1994). HBV is an essential cofactor in the evolution of hepatocellular damage (Hadziyannis, 1997 & Davies, 1992). Seroprevalence studies of anti-HD in HBsAg-positive patients has shown a worldwide but not uniform distribution (Purcell and Gerin, 1996).
The HDV was shown to rely on HBV for transmission because it uses the HBsAg as its own virion coat (Purcell and Gerin, 1996, Lai, 1994 and Rizzetto, 1977). The mode of transmission is parenteral. HDV infection has been reported in every geographical region of the world. Two epidemiologic patterns of HDV exist: in Mediterranean countries infection is endemic among HBV carriers and the virus is transmitted by close personal contact. In Western Europe and North America, HDV is confined to persons exposed to blood or blood products. Worldwide, more than 10 million people are infected with HDV (Hadziyannis, 1997). Traditional methods for the diagnosis of HDV infection, such as detection of serum anti-HDV antibodies are sufficient for the clinical diagnosis of HDV. HDV coinfection is associated with increased morbidity and patients with HBV should be tested for HDV infection (Cross Timothy et al., 2008).

Currently there is no effective antiviral therapy available for treatment of acute or chronic type D hepatitis (Purcell and Gerin, 1996). Interferon α has been tried therapeutically with only marginal and transient effects. Ribavirin has been shown to inhibit HDV RNA replication in tissue culture. Since HDV is dependent on HBV for replication, control of HDV infection is achieved by targeting HBV infections. All measures aimed at preventing the transmission of HBV will prevent the transmission of HDV. HBV vaccination is therefore recommended to avoid HBV-HDV coinfection (Lai, 1994). For infected patients, massive doses of α-interferon have yielded remissions but most patients remained positive for HDV RNA despite the improved disease conditions (Purcell and Gerin, 1996). The effect of interferon is considered to be most likely an indirect one, possibly via an effect on the helper hepadnavirus on the immune response to the infections (Taylor, 1996). Acyclovir, ribavirin, lamivudine and synthetic analogues of thymosin have proved ineffective (Hadziyannis, 1997). Immunosuppressive agents do not have any effect on HDV (Purcell, 1996 & Lai, 1994). Liver transplantation has been helpful for treating fulminant acute and end-stage chronic hepatitis (Purcell and Gerin, 1996). In one study the 5-year survival rate of transplant patients for terminal delta cirrhosis was 88% with reappearance of HBsAg only in 9% under long-term anti-HBs prophylaxis (Hadziyannis, 1997). No routine blood screening for HDV is currently performed however, HDV infection by contaminated blood sources can be prevented by screening for HBV.
A significant proportion of acute viral hepatitis occurring in young to middle-age adults in Asia, Africa and the Indian subcontinent is caused by an enterically transmitted viral agent that is serologically unrelated to HAV. Hepatitis E (HEV) was not recognized as a distinct human disease until 1980. The virus was first identified as the cause of extended waterborne outbreaks of hepatitis, with significant deaths among pregnant women (Khuroo, 1980). HEV was suggested to be classified in the Picornaviridae family (Balayan et al., 1983). However, later studies showed that it does not belong to members of this family. Between 1988 and 1998, HEV was tentatively classified in the Caliciviridae family, based on virion morphology. This classification was also rejected after a phylogeny analysis of the HEV genome and HEV was newly classified as an independent genus HEV-like virus unassigned to any family (Acha and Szyfres, 2003 & Berke and Matson, 2000). At present, HEV is the only member of the Hepevirus genus, Hepeviridae family (Emerson et al., 2004).

Various clinical manifestations of the disease have been observed from more frequent subclinical forms to fulminant forms of hepatitis. HEV infection is most often seen in children, young to middle aged adults (15 to 40 years old) and might be serious in pregnant women. In most cases, the signs and symptoms of the disease include moderately severe hepatitis with concurrent signs of influenza-like symptoms, abdominal pain, tenderness, nausea, vomiting and fever in the first (preicteric) phase of 1 to 10 days. The second (icteric) phase (15 to 40 days) with concurrent jaundice and dark urine is followed by viremia, liver enzyme elevations, antibody seroconversion and clearing of the virus. Clinically, HEV is typically a self-limiting disease without progression to chronic illness. HEV is mostly asymptomatic in children but fulminant hepatitis occurs more frequently during pregnancy with a mortality rate of 20% among pregnant women in the third trimester, and can also cause premature births. The reason for the high mortality of pregnant women is not fully determined (Emerson and Purcell, 2003, Worm et al., 2002, Bednar et al., 1999 and Hussaini et al., 1997).

HEV has a single-stranded positive-sense RNA genome of 7.2 kb. It consists of a short 5′ nontranslated region (27 to 35 nucleotides in length) followed by three partially overlapping forward open reading frames (ORFs, from 5′end ORF1, ORF3, ORF2). The 3′ nontranslated region is 65 to 74 nucleotides in length, terminated with a poly(A) end with 150 to 200 nucleotides in length. The 5′ end of the RNA is modified by m7G capping (Kabrane-Lazizi et al., 1999b). The HEV genome structure in all
human and animal strains is comparable. The exact mode of replication and expression of HEV has not been recognized yet. The assumed course of events has been mostly based on analogy with other viruses and on the knowledge of conservative segments of non-structural HEV domains (Worm et al., 2002).

HEV infections spread mainly by the faecal-oral route and large epidemics due to this virus are often associated with contaminated water (Vasickova et al., 2005, Ashbolt, 2004, Koopmans and Duizer, 2004). The primary cause of HEV outbreaks in developing countries is poor sanitation. Particularly during heavy rainfall, contamination of both drinking water supplies and coastal waters with human and animal faeces occurs (Balayan 1997, Tsega et al., 1991 and Balayan et al., 1983). The propensity for waterborne transmission suggests that shellfish could also become contaminated and thus acts as a vector for transmission of HEV. Although direct evidence is not currently available, the association of shellfish with transmission of HEV is highly suggestive (Lees, 2000). Cacopardo et al., (1997), consider a stay in a tropical zone and consumption of raw or undercooked shellfish as risk factor for HEV transmission. Person-to-person transmission of HEV between family members has been documented in only 1% to 2% of cases, in contrast to 15% person-to-person transmission of HAV (Khuroo, 1980). Transplacental transmission of HEV in the third trimester of pregnancy has been described; it is associated with a high perinatal mortality of the affected newborns (Bednar et al., 1999 & Khuroo et al., 1995).

HEV is classified as one of the water borne viruses and it could be regarded as both an emerging anthroponosis and zoonosis. Sporadic occurrence of HEV has been described in industrialised countries where affected people have been associated with a travel history to countries with an increased risk of infection (Dawson et al., 1992 & Skidmore et al., 1991). Two of the largest and most recent epidemics occurred in northwestern China between 1986 and 1988 with about 120 000 clinical cases reported in 1991 in Kanpur, India affecting 79 000 persons. Later studies reported HEV infections in people without a travel history to these countries (Smith, 2001 & Schlauder et al., 1998). Developing countries of Asia, Africa, South and Central America (countries with endemic occurrence of HEV) are considered as risk areas (Vasickova et al., 2005, Ashbolt, 2004, Koopmans and Duizer, 2004 and Hubalek, 2003). HEV is not endemic in the USA and parts of Western Europe. With few
exceptions, the confirmed cases of HEV have been traced to immigrants and tourists from countries where HEV is endemic. These ‘imported cases’ establish that HEV is a worldwide public health problem.

Nucleic acid-based techniques, especially nested RT PCR and real-time PCR have emerged rapidly as the method of first choice for sensitive and specific detection of RNA viruses. This method is very useful in research for the characterisation of divergent HEV strains whose serological responses have not been detected by some assays especially in countries where infection is not endemic (Worm et al., 2002, Schlauder et al., 1999, Wang et al., 1999 and Hsieh et al., 1998).

Recent research on the identification of an effective subunit vaccine for HEV has yielded encouraging results. At present, no commercially available vaccines exist for the prevention of HEV. There is no hyperimmune E globulin available for pre or post exposure prophylaxis. No available therapy is capable of altering the course of acute infection. As no specific therapy is capable of altering the course of acute HEV infection, prevention is the most effective approach against the disease. Hospitalization is required for fulminant hepatitis and should be considered for infected pregnant women.

Due to the fact that almost all HEV infections are spread by the faecal-oral route, good personal hygiene, high quality of standards for public water supplies and proper disposal of sanitary waste in developing countries have been recommended. For travellers to highly endemic areas, the usual elementary local food hygiene precautions are recommended. These include avoiding drinking water and/or ice of unknown purity as well as eating uncooked shellfish and uncooked fruits or vegetables that are not peeled or prepared by the travellers themselves.

**HCV**

HCV is a small (50 nm in size), enveloped, positive sense single strand RNA virus belongs to the family *Flaviviridae*. The structure of the HCV virus consists of a core of genetic material (RNA), surrounded by an icosahedral protective shell of protein, and further encased in a lipid (fatty) envelope of cellular origin. Two viral envelope glycoproteins, E1 and E2 are embedded in the lipid envelope (Op De Beeck
and Dubuisson, 2003). HCV has a positive sense RNA genome that consists of a single open reading frame of 9600 nucleoside bases (Kato, 2000). At the 5' and 3' ends of the RNA there are regions that are not translated into proteins but are important to translation and replication of the viral RNA. The 5' UTR has a ribosome binding site (Jubin, 2001) that starts the translation of a 3000 amino acid containing protein that is later cut by cellular and viral proteases into 10 active structural and non-structural smaller proteins (Dubuisson, 2007).

Replication of HCV involves several steps. The viruses need a certain environment to be able to replicate, and must therefore first move to such areas. HCV has a high rate of replication with approximately one trillion particles produced each day in an infected individual. Due to lack of proofreading by the HCV RNA polymerase, HCV also has an exceptionally high mutation rate, a factor that may help it elude the host's immune response.

HCV mainly replicates within hepatocytes in the liver, although there is controversial evidence for replication in lymphocytes or monocytes. By mechanisms of host tropism, the viruses reach these proper locations. Circulating HCV particles bind to receptors on the surfaces of hepatocytes and subsequently enter the cells. Two putative HCV receptors are CD81 and human scavenger receptor class B1 (SR-BI). However, these receptors are found throughout the body. The identification of hepatocyte-specific cofactors that determine observed HCV liver tropism are currently under investigation. Once inside the hepatocyte, HCV initiates the lytic cycle. It utilizes the intracellular machinery necessary to accomplish its own replication (Lindenbach and Rice, 2005).

**Taxonomy**

HCV has been classified as the sole member of a distinct genus called hepacivirus in the family *Flaviviridae*, which includes the flaviviruses, the animal pathogenic pestiviruses.
Characteristic features

HCV is usually detectable in the blood within one to three weeks after infection and antibodies to the virus are generally detectable within 3 to 12 weeks. The first six months after infection with HCV refers to acute hepatitis. During the acute phase between 60% to 70% of people infected develop no symptoms. Symptoms of acute HCV infection include decreased appetite, fatigue, abdominal pain, jaundice, itching, and flu-like symptoms. Approximately 15 - 40% of persons infected with HCV clear the virus from their bodies during the acute phase as shown by normalization in liver function tests (LFTs) such as Alanine transaminase (ALT) and Aspartate transaminase (AST) normalization, as well as plasma HCV-RNA clearance known as spontaneous viral clearance. The remaining 60 - 85% of patients infected with HCV develops chronic hepatitis C, i.e., infection lasting more than 6 months (Cox et al., 2005, NIH, 2002 and Villano et al., 1999).

The natural course of chronic hepatitis C varies considerably from one person to another. Chronic hepatitis C is defined as infection with the hepatitis C virus persisting for more than six months. Clinically, it is often asymptomatic and it is mostly discovered accidentally. Virtually all people infected with HCV have evidence of inflammation on liver biopsy. However, the rate of progression of liver scarring shows significant variability among individuals. Recent data suggests that among untreated patients, roughly one-third progress to liver cirrhosis in less than 20 years. Another third progress to cirrhosis within 30 years. The remainder of patients appear to progress so slowly that they are unlikely to develop cirrhosis within their lifetimes. Factors that have been reported to influence the rate of HCV disease progression include age (increasing age associated with more rapid progression), gender (males have more rapid disease progression than females), alcohol consumption (associated with an increased rate of disease progression), HIV coinfection (associated with a markedly increased rate of disease progression) and fatty liver (the presence of fat in liver cells has been associated with an increased rate of disease progression).

HCV is a systemic disease and patients may experience a wide spectrum of clinical manifestations ranging from an absence of symptoms to a more symptomatic illness prior to the development of advanced liver disease. Symptoms specifically suggestive of liver disease are typically absent until substantial scarring of the liver has
occurred. Generalized signs and symptoms associated with chronic HCV include fatigue, marked weight loss, flu-like symptoms, muscle pain, joint pain, intermittent low-grade fevers, itching, sleep disturbances, abdominal pain (especially in the right upper quadrant), appetite changes, nausea, diarrhea, dyspepsia, cognitive changes, depression, headaches, and mood swings.

Once chronic HCV has progressed to cirrhosis, signs and symptoms may appear that are generally caused by either decreased liver function or increased pressure in the liver circulation, a condition known as portal hypertension. Possible signs and symptoms of liver cirrhosis include ascites (accumulation of fluid in the abdomen), bruising and bleeding tendency, bone pain, varices (enlarged veins, especially in the stomach and esophagus), fatty stools (steatorrhea), jaundice and a syndrome of cognitive impairment known as hepatic encephalopathy. Chronic HCV more than other forms of hepatitis is diagnosed because of extrahepatic manifestations associated with the presence of HCV such as thyroiditis (inflammation of the thyroid) with hyperthyreosis or hypothyreosis, porphyria cutanea tarda, cryoglobulinemia (a form of vasculitis) (Pascual et al., 1990) and glomerulonephritis (inflammation of the kidney), specifically membranoproliferative glomerulonephritis (MPGN) (Johnson et al., 1993). HCV is also associated with sicca syndrome, thrombocytopenia, lichen planus, diabetes mellitus and with B cell lymphoproliferative disorders (Zignego et al., 2006).

Molecular biology

HCV genome carries a single long open reading frame (ORF) encoding a polypeptide that is proteolytically cleaved into a set of distinct products. Translation of the HCV ORF is directed via a 340 nucleotide long 5’ non-translated region (NTR) functioning as an internal ribosome entry site (IRES) and permitting the direct binding of ribosomes in close proximity to the start codon of the ORF (Wang et al., 1993 & Tsukiyama-Kohara et al., 1992). The first 40 nucleotides of the RNA genome are not required for translation but based on analogy with other plus-strand RNA viruses involve most likely in RNA replication (Boyer and Haenni, 1994). The 3’ NTR was only recently discovered (Kolykhalov et al., 1996, Yamada et al., 1996, Tanaka et al., 1996 and 1995). It has a tripartite structure composed of variable sequences following the stop codon of the ORF, a poly (U) tract of heterogeneous length and a highly
conserved 98 nucleotide sequence essential for replication in-vivo (Kolykhalov et al., 2000 & Yanagi et al., 1999).

The HCV polyprotein is cleaved co- and post-translationally by cellular and viral proteinases into ten different products, with the structural proteins located in the aminoterminal end. The first cleavage product of the polyprotein is the highly basic core protein, forming the major constituent of the nucleocapsid (Yasui et al., 1998). In addition, a number of other functions like modulation of several cellular processes or induction of HCC in transgenic mice have been described (Chang et al., 1998, Moriya et al., 1998, Chen et al., 1997 and Matsumoto et al., 1997). Envelope proteins (E1 and E2) are highly glycosylated type 1 transmembrane proteins, forming two types of stable heterodimeric complexes: a disulfide-linked form representing misfolded aggregates and a non-covalently linked heterodimer corresponding most likely to the pre-budding complex (Deleersnyder et al., 1997). In addition, E2 was shown to interact with the IFN-induced double-stranded RNA-activated protein kinase PKR. Upon induction by IFN-α, this enzyme reduces protein synthesis via phosphorylation of translation initiation factor eIF2-α, but in cells containing E2, PKR is inhibited, allowing continuation of translation in the presence of IFN (Taylor et al., 1999). Protein p7, located at the carboxy terminus of E2, is a highly hydrophobic polypeptide of unknown function. Most of the nonstructural (NS) proteins 2 - 5B are required for replication of the viral RNA (Lohmann et al., 1999b). NS2 and the amino-terminal domain of NS3 constitute the NS2-3 proteinase, catalysing cleavage at the NS2/3 site (Grakoui et al., 1993a, Hijikata et al., 1993a and Hirowatari et al., 1993). NS3 is a bifunctional molecule carrying the amino-terminal C180 residues, a serine-type proteinase responsible for cleavage at the NS3/4A, NS4A/B, NS4B/5A and NS5A/B sites and in the carboxy-terminal remainder, NTPase/helicase activities essential for translation and replication of the HCV genome (Kolykhalov et al., 2000, Gwack et al., 1996, Hong et al., 1996, Tai et al., 1996, Kim et al., 1995, Bartenschlager et al., 1993, Eckart et al., 1993, Grakoui et al., 1993b, Suzich et al., 1993 and Tomei et al., 1993). In addition, NS3 may have other properties involved in interference with host cell functions like inhibition of protein kinase A-mediated signal transduction or cell transformation (Borowski et al., 1996 & Sakamuro et al., 1995). NS4A is an essential cofactor of the NS3 proteinase and is required for efficient polyprotein processing (Tanji et al., 1995b, Bartenschlager et al., 1994, Failla et al., 1994 and Lin et al., 1994b). The function of
the hydrophobic NS4B is so far unknown. NS5A is a highly phosphorylated protein and, at least with some HCV isolates, the level of phosphorylation is influenced by NS4A via direct interaction with NS5A or it requires the expression of NS5A in the context of a NS3-5A polyprotein (Koch and Bartenschlager, 1999, Neddermann et al., 1999, Asabe et al., 1997, Tanji et al., 1995b and Kaneko et al., 1994). NS5A phosphorylation is mediated by an as yet unknown cellular kinase (Reed and Ria, 1997 and Tanji et al., 1995b). For the HCV-H isolate the major phosphorylation site has been mapped to serine residue 2321 of the polyprotein and the proline-rich nature of the flanking sequence suggests that a proline-directed kinase is responsible for NS5A phosphorylation (Reed and Rice, 1999). The role NS5A may play in RNA replication is so far not known, but based on analogy with other RNA viruses, where phosphoproteins are important regulators of replication one could assume that NS5A plays a similar role. Apart from such a function, NS5A appears to be involved in resistance of the infected cell to the antiviral effect of IFN. At least for some HCV isolates NS5A is able to bind to PKR, blocking the translational reduction in the IFN-treated cell (Gale et al., 1998 & 1997). Interestingly, an alanine substitution for the major phosphorylation site at serine residue 2321 did not affect the NS5A: PKR interaction, showing that phosphorylation at this particular site is not required for complex formation with PKR (Reed and Rice, 1999). NS5B was identified as the RNA dependent RNA polymerase (Al et al., 1998, Yamashita et al., 1998, Lohmann et al., 1997 and Behrens et al., 1996).

Transmission

Injection drug use is the most important source of HCV transmission in the developed world, accounting for approximately 2/3 of infections in the US and Western Europe and as much as 80% in Australia (Shepard et al., 2005, Dore et al., 2003 and Alter, 2002). The practice of unsafe therapeutic injection in the developing world has emerged as an important source of transmission in those parts of the world. An important example is transmission by way of contaminated reusable glass syringes in Egyptian schistosomiasis treatment programmes, leading to that nation’s very high seroprevalence (Frank et al., 2000). A community-based screening program in Soviet Union found that high prevalence of HCV infection resulted from inadequately sterilized medical equipment. Blood transfusion has diminished in importance as a risk of HCV transmission in the developing world since the institution of all volunteer
donation and effective screening methods (Seeff et al., 1975). It is likely that blood products remain a significant reservoir of HCV in developing nations (CDC, 1998). Other potential controversial exposures include tattooing and body piercing, intranasal cocaine use and high-risk sexual practices. Recognition of HCV transmission risk factors has led to a decrease in the incidence of new infections especially in developed nations but the prevalence of chronic HCV is expected to rise over the coming decades (Alter et al., 1994).

Equipment that may harbor contaminated blood if improperly sterilized includes needles or syringes, hemodialysis equipment, oral hygiene instruments, jet air guns etc. can also transmit HCV. Scrupulous use of appropriate sterilization techniques and proper disposal of used equipment can reduce the risk of iatrogenic exposure to HCV to virtually zero. Contact sports and other activities such as "slam dancing" that may result in accidental blood-to-blood exposure are potential sources of exposure to HCV (Karmochkine et al., 2006). Sexual transmission of HCV is considered to be rare. Tattooing dyes, ink pots, stylets and piercing implements can transmit HCV-infected blood from one person to another if proper sterilization techniques are not followed. Tattoos or piercings performed before the mid 1980s, "underground," or non-professionally are of particular concern since sterile techniques in such settings may have been or be insufficient to prevent disease. Personal care items such as razors, toothbrushes, cuticle scissors and other manicuring or pedicuring equipment can easily be contaminated with blood. Sharing such items can potentially lead to exposure to HCV.

Vertical transmission refers to the transmission of a communicable disease from an infected mother to her child during the birth process. Mother-to-child transmission of HCV has been well described, but occurs relatively infrequently. Transmission occurs only among women who are HCV RNA positive at the time of delivery and the risk of transmission in this setting is approximately 6 out of 100. Among women who are both HCV and HIV positive at the time of delivery the risk of HCV is increased to approximately 25 out of 100. The risk of vertical transmission of HCV does not appear to be associated with method of delivery or breast feeding.
Pathogenesis

The immune response has an unique role in the pathogenesis of viral hepatitis because it contributes both to viral infection control and healing as well as in developing chronic infection and liver cirrhosis. HCV is a non-cytopathic hepatotropic virus that induces acute or chronic liver disease and interacts in a complex way with the immune system (Rehermann and Nascimbeni, 2005). The immune response (innate and adaptive) represents the first line of defense against viral replication, HCV has complex mechanisms to elude this immune response. Interactions between HCV and host immune response in the first week after exposure may substantially influence the subsequent evolution and the prognosis of infection (Bertoletti and Ferrari, 2003). One-to-two weeks after exposure, HCV-RNA can be detected in serum and it quickly replicates, reaching serum levels of around 106 copies/ml (Hoofnagle, 2002). However, immunology studies showed a delay of the cellular adaptive immune response of 1 - 2 months (Bertoletti and Ferrari, 2003) and of the humoral response of 2 - 3 months (Rehermann and Nascimbeni 2005). These observations led to the hypothesis that HCV manages to surpass the adaptive immune response. This hypothesis is backed up by the rarity of symptomatic C virus infections, as we know that clinical signs and especially jaundice are caused by liver injuries mediated by T lymphocytes (Rehermann and Nascimbeni, 2005). Another observation is that in HCV infection, the adaptive immune response seems to ignore significant viral levels for several weeks while in HBV infection the limited HBV antigen levels (in the early stages of infection) seem to be responsible for delaying the adaptive immune response (Bertoletti and Ferrari, 2003). After the first week from exposure, the initial peak of viral replication is followed by a period of 4 - 6 weeks during which HCV-RNA may slightly elevate or remain stable, in the absence of specific HCV B and T lymphocytes and liver inflammation induction (Hoofnagle, 2002). Serum aminotransferase levels begin to rise 2 - 8 weeks after exposure, and at 8 - 12 weeks, when their levels reach the maximum value HCV-RNA levels diminish. Anti HCV antibodies presence is variable becoming detectable at the time of aminotransferases peak, later or not at all. Viral clearance can occur before a measurable humoral response or even in its absence therefore a small proportion of patients can have negative EIA tests for anti HCV antibodies during the acute phase and heal without developing any detectable serologic marker of infection (Heller and Rehermann, 2005). More frequently, antibodies levels fall down in time after the viral clearance and may even disappear at 10 - 20 years after healing; this situation is
encountered in 7 - 40% patients with spontaneous clearance and those have no serologic marker of previous infection (Heller and Rehermann, 2005 & Hoofnagle, 2002). Viral clearance from the liver and possibly from other pools takes more time than the serum clearance-hypothesis sustained by the recurrence of viremia after several months of undetectable values. At the same time, there is a debate whether HCV is completely eradicated (Rehermann and Nascimbeni, 2005).

**Epidemiology**

HCV continues to be a major disease burden world wide. In 1999, the WHO estimated a prevalence of about 3% with the virus affecting 170 million people worldwide (WHO, 1999). Generally, most studies of prevalence use blood donors to report the frequency of HCV usually by anti-HCV antibodies and do not report follow-up HCV testing. Using blood donors as a prevalence source may underestimate the real prevalence of the virus because donors are generally a highly selected population (Alter et al., 1999).

Among Central and South America, a recent community based study in San Juan, Puerto Rico, showed that estimated prevalence of HCV in 2001 - 2002 was 6.3% (Perez et al., 2005). In Mexico, the prevalence reported was about 1.2%. Among blood donors in Chile and Brazil, prevalence of HCV Ab was low-0.3%, 1.14% respectively (Munoz et al., 1998 & Vasconcelos et al., 1994).

In Europe, general prevalence of HCV is about 1% but varies among the different countries (Touzet et al., 2000). Prevalence of HCV antibody is 0.87% (1993 - 1994) in Belgium (Van Damme et al., 2002). In the United Kingdom, at least 200,000 adults carry HCV. In Northern Italy, prevalence of HCV Ab was 3.2% (Bellentani and Tiribelli, 2001). Three studies in Central and Southern Italy showed a higher rate of HCV (8.4% - 22.4%), especially in the older population (Raffaele et al., 2001, Maio et al., 2000 and Stroffolini et al., 1995). Among patients of general practitioners in Lyon, France the prevalence of HCV was estimated to be 1.3%, very similar to the French general population (Pradat, 2001). Within the Russian army, frequency of anti-HCV was 1.5% among servicemen and donors with increased prevalence in the North Caucasus, Far East and Siberia (3.1 - 3.8%) compared to the Transbaikal region (0.7%)
(Ogarkov et al., 2004). Low rates were found in Hungary (0.73% of 15,864 blood donors) (Barna et al., 1996).

There is a paucity of information on HCV prevalence in developing countries, especially in Asia and Pacific regions, where the largest segment of population resides (Poovorawan et al., 2002, Debonine et al., 1999 and Sun et al., 1999). Several reports on adults in the Western World suggest that the epidemiology of HCV infection is changing, both in the general population and in selected risk groups due to intercurrent socio-sanitary events, such as HCV screening of blood donors, HIV and HCV prevention campaigns, changing patterns of drug abuse and efficient therapy for eradicating the infection (Ansaldi et al., 2005, Gerard et al., 2005, Shepard et al., 2005, Mazzeo et al., 2003, Matera et al., 2002, Schroter et al., 2002, Bellentani et al., 2000 and Gonzales et al., 2000). World wide the prevalence of HCV is found to be high in Egypt from 6 to 28% (Kowo et al., 1995, Farrell et al., 1993 and Tanaka et al., 1992).

Recently, HCV prevalence studies have come out of Pakistan in the Middle East. 751 out of 16,400 patients (4.57%) were found to be positive for HCV Ab from 1998 - 2002 with the largest age group from 41 - 50 (Muhammad and Jan, 2005). Among male blood donors in Karachi, Pakistan the seroprevalence of HCV was 1.8% with a trend of increasing proportion of positive donors from 1998 - 2002 (Aktar et al., 2004). There has been very high prevalence rates of HCV reported in Egypt in the past (28%) (Saeed et al., 1991). This was confirmed among 90 blood donors in Cairo, where 14.4% were anti HCV positive by RIBA test (Darwish et al., 1992). Then 26.6% among 188 blood donors and 22% among 163 donors were positive with both studies done in Cairo (Bassily et al., 1995 & Darwish et al., 1993). Rates were lower in Saudi Arabia (1.8%) and Yemen (2.1%) (Al-Faleh et al., 1995 & El Guneid et al., 1993).

Intermediate rates of HCV have been reported out of Asia. From 1995 - 2000, 0.49% anti-HCV Ab were detected among 3,485,648 blood donors in Japan (Tanaka, 2000). This was lower than the 0.98% in 1992 (Yamaguchi et al., 1994). In China, prevalence rates were generally low with rates around 1% among donors in Beijing and Wuhan (Wang et al., 1994 & Zhang et al., 1992). However, rates may be higher in certain areas such as the Hubei province (30.13%) and Inner Mongolia Autonomous Region (31.86%) (Tang, 1993). Low rates have been found in Malaysia (around 1.6%)
and Singapore (0.54%). Higher rates of HCV have been found in Thailand (3.2 - 5.6%) (Apichartpiyakul et al., 1999 & Songsivilai et al., 1997). Within a smaller community of 103 residents in Sherpas, Nepal only 1 person had a borderline reaction in 2004 (Chiba et al., 2004). In New Delhi, India 1.85% of blood donors were positive for HCV (Panigrahi et al., 1997). There have been fewer studies out of Africa, but lower rates have been reported-1.6% among blood donors in Ethiopia and 0.9% in Kenya (Ilako et al., 1995 & Frommel et al., 1993). The estimated prevalence in Australia has been recently reported as 2.3% with the virus affecting 210,000 people by 2001. The 20 - 24 year old age group had the highest prevalence with strong majority of the infected population below the age of 50 (Amin et al., 2004).

**Risk Factors**

**Intravenous drug use**

Transmission of HCV virus has been strongly associated with intravenous and percutaneous drug and needle use. Reported cases of HCV from intravenous drug use is on the rise in the US. In a study of injection drug users in Baltimore, Maryland from 1988 to 1996, 30.3% of participants developed anti-HCV antibodies with most in the first 2 years of the study (Villano et al., 1997). Among 310 drug users in Antwerp and Limburg in Belgium, 71% and 46% had anti-HCV antibody, respectively (Mathei et al., 2005). The Hepatitis C European Network for Co-operative Research (HENCORE) group reported a prevalence of HCV of 80% among intravenous drug users (IVDU) (Touzet et al., 2000). In a study in Pakistan, all 751 anti-HCV patients had a history of injections (Muhammad and Jan, 2005). 90% of IVDU in Chang Rai, Thailand were positive for HCV (Apichartpiyakul et al., 1999). A study conducted in Glasgow demonstrates that Women who engage in street sex work to finance their drug habit show HCV infection risk greater than that for other IDUs (Avril Taylor et al., 2008). 36.6% of randomly selected IVDU in Sydney, Australia and 74% of IVDU in Melbourne, Australia were HCV positive (Bradshaw et al., 2005 & Maher et al., 2004). A recent study in London, England took 428 intravenous drug users below the age of 30 and found that 44% had antibodies to HCV compared to 4% with HIV. This came out to an incidence of 41.8 cases per 100 person positive for antibody to HCV (Judd et al., 2005).
The importance of intravenous drug use can not be overemphasized. The prevalence of HCV among people who acquired HIV through intravenous drug use reaches 90% (Sulkowski and Thomas, 2003). Incidence of HCV infection among injection drug users in the United States decreased from 1994 through 2004 (Amon et al., 2008). Coinfection of the two viruses can make treatment all the more difficult. Most countries with a young population of HCV infection must deal with intravenous drug use as the leading cause for spread of the virus. Many of these intravenous drug users do not know they are infected. Screening of HCV and treatment of substance abuse are extremely important in this group.

Blood Transfusions

Transfusion of blood products has been a leading cause of transmission of HCV. However, due to improved screening, transmission through transfusions has decreased in most developed countries. In Japan, incidence of post-transfusion non-A non-B hepatitis among those with less than 10 transfusions dropped from 4.9% (1988-Oct '89) to 1.9% (Nov 89 - 90) after screening with first-generation anti HCV test was introduced. In the US, incidence of post-transfusion HCV dropped from 3.84% to 0.57% per patient (0.03% per unit blood) after HCV screening was introduced in 1990 (Donahue et al., 1992). In England, the frequency HCV infected donations dropped from 1 in 520,000 (1993 - 98) to 1 in 30 million (1999 - 2001) when donations were tested for HCV RNA (Soldan et al., 2003).

However, incidence of transfusion related HCV is still higher in other areas of the world. In a study of 147 Chilean patients with chronic HCV, the most common risk factor was blood transfusion in 54% versus just 5% with IVDU (Soza et al., 2004). A study was done in the largest blood bank in Santa Catarina, Brazil from 1991 - 2001 showing a significant drop in risk of acquiring HCV, 10 times higher than that of developed countries (Kupek, 2004). Despite better screening for selecting blood donors, there remains a need for some kind of HCV screening laboratory test.
Sexual activity

The role of sexual activity in the transmission of HCV remains unclear. In the NHANESIII study, number of sexual partners and age at first sexual intercourse had significant correlation with HCV Ab and this has been confirmed in other studies (Perez et al., 2005 & Alter, 1999). Among 1257 non-IVDU in Baltimore at a STD clinic 9.7% were positive for HCV (Thomas et al., 1994). One hypothesis is that many of the HCV patients may have injecting sexual partners. In one study, 15% of non IVDU women with an injecting partner had HCV (Goldberg et al., 2001). More recently, a 10-year prospective follow-up study (8060 person-years) showed no evidence of sexual transmission among monogamous couples in Italy (Vandelli et al., 2004). However, in a study among spouses in Egypt, it was estimated that wife to husband transmission was 34% and 10% among women with and without detectable HCV RNA. Husband to wife transmission was estimated at 3%. Overall, 6% were estimated to have contracted HCV from their spouse (Magder Fix et al., 2005). Recently, there was lack of evidence found for sexual transmission of HCV among men who have sex with men in the prospective ongoing Omega Cohort Study in the US (2653 person-years of follow-up) (Alary et al., 2005). All of this new evidence supports that sexual transmission of HCV is still rare but for some reason is higher among those with high-risk sexual activity.

Hemodialysis

It has been well documented that dialysis patients have a higher rate of HCV infection. In the 90's much of the world reported anti HCV prevalence rates of 10 - 50% among hemodialysis patients with lower rates in such places as Ireland (1.7%) (Hayashi et al., 1994, Conlon et al., 1993, Medin et al., 1993, Niu et al., 1993, Nordenfelt et al., 1993 and Hardy et al., 1992). Previously, rates in Europe were as high as 20 - 30% (Touzet et al., 2000). A more recent report from Saudi Arabia showed a prevalence rate of HCV among hemodialysis patients to be 9.24% compared to 0.30% among blood donors (Qadi et al., 2004). In a tertiary-care hospital in Mexico City, Mexico, the rate of anti-HCV was 6.7% compared to the roughly 1.2% prevalence in the population of Mexico (Mendez-Sanchez et al., 2004). The rate of seroconversion among hemodialysis patients with no other risk factors has been reported to be 1.38 - 1.9% per
year (Fabrizi et al., 1999 & Halfon et al., 1998). These studies generally conclude that the transmission of the virus to hemodialysis patients is generally nosocomial with possible risk factors being failure to disinfect devices between patients, sharing of single-use vials for infusions, poor sterile technique, poor cleaning of dialysis machines, and poor distance between chairs (Zampieron et al., 2004).

**Special Populations**

The prevalence of HCV has been noted to be higher in other populations as well. Among kidney transplants, the prevalence was reported to be as high as 33.3% in Italy with the frequency higher prior to 1990 (50%) than after 1990 (27%) (Angelico et al., 1997). Most of these kidney transplant patients underwent dialysis as well. The United States Veteran Affairs medical centers have also reported a higher prevalence of HCV than the general population with percentages as high as 35% in the VA Palo Alto system (Cheung, 2000). The most recent study among 20 centers reported an estimated prevalence of 5.4% with 78% reporting a risk factor of either transfusion or intravenous drug use. Seropositivity was also associated with tattoo use and incarceration in this study (Dominitz et al., 2005). There is also an increased prevalence of HCV among prison inmates. One example is the Riverside county jail system where 25% adults carried the virus while only 2% of the juvenile detention population carried HCV (Feldman et al., 2004). The juvenile detention population therefore provides a target for teaching and intervention since many of these juveniles acquire the virus early in their adult years.

**Diagnosis**

The diagnosis of HCV infection can be made by detecting anti HCV. Detection of anti HCV is recommended for routine testing of asymptomatic persons. Diagnosis of HCV infection can also be made through detection of HCV RNA using reverse transcriptase polymerase chain reaction (RT PCR) techniques. HCV RNA can be detected within one to two weeks after exposure to the virus, weeks before the onset of ALT elevations or the appearance of anti HCV (Young et al., 1995). In some patients, the detection of HCV RNA may be the only evidence of HCV infection.
Although polymerase chain reaction (PCR) assays for HCV RNA are available from several commercial laboratories on a research use basis, the results may vary considerably between laboratories. Both false-positive and false-negative results can result from improper collection, handling and storage of test samples. In addition, HCV RNA may be detected intermittently during the course of infection, so a single negative PCR result is not conclusive. Because of assay variability, rigorous proficiency testing is recommended and results of PCR testing should be interpreted cautiously (Gretch, 1997).

Quantitative assays for measuring the titer of HCV RNA have been developed, including a branched chain DNA assay and a quantitative PCR. Several different nucleic acid detection methods also have been developed to group isolates of HCV based on genotypes. Relatively few patients seek medical care for acute HCV, since most patients are asymptomatic or have only mild, flu-like symptoms. Of those who do present with acute HCV, 70 to 80 percent have detectable anti HCV at clinical presentation and 90 percent have detectable anti HCV by 12 weeks after onset (Vallari et al., 1992). Therefore, anti HCV testing should be repeated if acute HCV is suspected and the initial test result is negative. Most patients with acute HCV remain chronically infected, and approximately two thirds or more of patients with chronic infection have abnormal ALT activity (NIH, 1997).

In most instances, evidence of chronic HCV infection is discovered by chance through screening tests at the time of blood donation or a routine physical examination. Most persons who are found to be positive for anti HCV in these situations are chronically infected. No tests are available to differentiate acute, chronic and resolved infections and the diagnosis of chronic HCV is usually based on the presence of elevated ALT values in patients who are positive for anti HCV. For anti HCV positive patients with a normal ALT value, the presence of ongoing liver inflammation should be assessed by monitoring serum ALT values several times over six to 12 months because abnormalities may be present only intermittently in patients with chronic HCV (Young et al., 1995).
Treatment

In chronic HCV carriers there is a very small chance of clearing the virus spontaneously (Scott et al., 2006 & Watanabe et al., 2003) however the majority of patients with chronic HCV will not clear it without treatment. Therapy for HCV infection was first reported in the late 1980s when patients with so-called non-A, non-B hepatitis were treated with interferon (Hoofnagle et al., 1986). Treatment at that time was a monotherapy with 3 - 6 million with standard interferon alfa given three times per week subcutaneously for 24 or 48 weeks. Sustained virological response (SVR) rates were 2 and 7% for patients with genotype 1 infection treated for 24 and 48 weeks, respectively. For genotypes 2 and 3, a SVR was achieved in 16% (24 weeks) and 29 - 33% (48 weeks) (McHutchison et al., 1998 & Poynard et al., 1998). The introduction of combination therapy with interferon alfa and the nucleotide-analogon ribavirin (for 48 weeks) in the late 1990s led to a remarked improvement of therapy results with mean SVR rates of 41%. SVR for patients with genotype 1 (Hadziyannis et al., 2004, Fried et al., 2002 and Manns et al., 2001) were 28 - 36% compared to 61 - 79% for patients with genotypes 2 or 3 infection (Fried et al., 2002, Manns et al., 2001, McHutchison et al., 1998 and Poynard et al., 1998). Current treatment is a combination of pegylated interferon alpha (brand names Pegasys and PEG - Intron) and the antiviral drug ribavirin for a period of 24 or 48 weeks, depending on genotype. There are two formulations of this long-acting interferon which have roughly comparable effectiveness rates. In general, response rates are higher in those patients with genotypes other than type 1, weigh less, younger and have less fibrosis on liver biopsy (Ferenci, 2004). Treatment success is usually implied by achieving a sustained virological response (SVR), defined as lack of detectable virus in the serum 6 months following a treatment course. In registration trials peginterferon/ribavirin resulted in a SVR of 54%-56% (Fried et al., 2002 & Manns et al., 2001). Retrospective analysis has suggested response rates in excess of 60% in those patients who are able to take greater than 80% of their recommended dose of therapy for more than 80% of the recommended duration of treatment (McHutchison et al., 2002). Unnecessary prolongation of treatment in those patients destined to be viral nonresponders can be facilitated by measurement of viral load early in therapy (Ferenci et al., 2005). The current standard time for such measurement is at 12 weeks of treatment, but earlier “stopping rules” are under investigation (Zeuzem et al., 2005). Higher rates of SVR (76% - 82%) are seen in those patients who are infected with HCV.
genotypes 2 and 3, even when using some what lower ribavirin doses for a more abbreviated treatment course (Hadziyannis et al., 2004, Fried et al., 2002 and Manns et al., 2001). In addition to the standard treatment with interferon and ribavirin, some studies have shown higher success rates when the antiviral drug amantadine (Symmetrel) is added to the regimen. Sometimes called "triple therapy", it involves the addition of 100 mg of amantadine twice a day. Studies indicate that this may be especially helpful for "nonresponders"-patients who have not been successful in previous treatments using interferon and ribavirin only (Maynard et al., 2006). Currently, amantadine is not approved for treatment of HCV and studies are ongoing to determine when it is most likely to benefit the patient. Follow up studies have shown no benefit to adding this drug and currently it is not commonly used by experienced hepatologists.

The development of pegylated (PEG) interferons with sustained absorption a slower rate of clearance and a longer half-life than unmodified interferons, led to a further improvement of virological response rates especially for genotype 1 infected patients. Since 2001, the combination of PEG-interferon alfa and ribavirin has been established as standard therapy for chronic HCV with an average SVR rate of 54 - 63% (Hadziyannis et al., 2004, Fried et al., 2002 and Manns et al., 2001).

Due to a frequent lack of clinical symptoms, acute HCV is rarely diagnosed. After infection with HCV, merely 20 - 25% of patients show clinical evidence of acute (icteric) hepatitis. An icteric occurs of acute HCV and infection with HCV genotype 3 seems to be associated with higher rates of spontaneous recovery (Lehmann et al., 2004 & Gerlach et al., 2003). In patients with an icteric course of acute HCV, spontaneous recovery rates of more than 50% were reported (Gerlach et al., 2003, Hofer et al., 2003 and Alberti et al., 2002). Treatment of acute HCV with interferon was shown to be safe and effective, leading to SVR rates up to 98% after 24 weeks of therapy with standard interferon alfa (Gerlach et al., 2003 & Jaeckel et al., 2001).

In 50 - 70% of patients, HCV infection becomes chronic with detectable HCV RNA for more than 6 months after infection. Diagnosis of chronic HCV is established on the basis of detectable HCV RNA over more than 6 months after infection. The primary goal of antiviral therapy in patients with chronic HCV is a sustained
virological response. Long-term analysis shows that a relapse after 12 - 24 weeks of follow-up is rare (Veldt et al., 2004 & Zeuzem et al., 2003). SVR is accompanied by a significant reduction of inflammatory activity and fibrosis in the liver (Poynard et al., 2000). The indication for antiviral therapy in patients with chronic HCV is based on liver function tests, histological grading and staging of liver damage, extra hepatic manifestations of the disease, the social and personal situation of the patient as well as the risk of transmission to third persons (Fleig et al., 2004 & Zeuzem, 2004). Previously, therapy was only indicated for patients with elevated aminotransferase levels (National Institute of Health, 2002). A recently published study shows that the efficacy and safety of PEG-interferon/ribavirin combination therapy are similar in patients with persistently normal aminotransferase levels to patients with elevated liver enzymes. In approximately 50% of patients with normal ALT levels at baseline, intermittent elevations of liver enzymes were seen during 72 weeks of observation (Zeuzem et al., 2004).

Prevention

Primary prevention of HCV should target reduction of transmission of the virus. Prevention should target those at risk of acquiring the virus and should involve providing education, risk reduction counseling, HCV screening and substance abuse treatment. In the US, the Centers for Disease Control (CDC) suggest screening for the following population:

- Persons who ever injected illegal drugs, including those who injected once or a few times many years ago.
- Persons who received a blood transfusion or organ transplant before July 1992.
- Persons who received clotting factor concentrates before 1987.
- Persons who were ever on long-term dialysis.
- Children born to HCV positive women.
- Healthcare, emergency medical and public safety workers after needlesticks, sharps, or mucosal exposures to HCV positive blood.
- Persons with evidence of chronic liver disease.

Extra attention should be given to populations in specific settings such as correctional institutions, drug treatment programs, programs for high risk youth, HIV counseling and testing sites, and STD clinics. In these settings, physicians should
always screen for intravenous drug use. As intravenous drug use remains the most important source of HCV transmission in the US and Europe, education within this group is an important preventive tool. In fact, it was behavior change in this cohort that was apparently responsible for the reduction of HCV incidence in the 1990s (Alter, 2002). Other potential sites for transmission reduction include the penal system, where high incidences is regularly reported. Vertical transmission of HCV has been reported to occur approximately in 4% of births (Mast et al., 2005 & Syriopoulou et al., 2005) but neither type of delivery nor breast feeding appear to be associated with transmission (European Paediatric Hepatitis C Virus Network, 2005). To date, routine screening of pregnant women for HCV infection has not been adopted in the US. When evaluating a patient newly diagnosed with HCV it is recommended to advice against sharing of razors and tooth brushes but to reassure regarding casual contact (National Institutes of Health, 2002). Sexual transmission is thought to occur but to be inefficient compared to HBV. Changing of sexual practices, such as the adoption of barrier methods of contraception is not recommended in long term monogamous couples in which one partner is HCV infected (CDC, 1998). Alcohol use should be limited, given the described synergistic effects between alcohol and HCV infection (Wiley et al., 1998).

Unlike HIV, HCV is found in high concentrations in filters, spoons and rinsing liquids that may be used in association with needle drug use. Patients should be counseled on contaminated equipment being a source of infection. Addiction care and counseling should be focused on with possible referrals for psychotherapy and detoxification (Backmund et al., 2005 & Edlin et al., 2005). Prevention in healthcare setting should also take place by having better sterilization, safer injections, reducing opportunities for percutaneous exposures to blood. In developing countries, better screening for donors and blood screening should take place to reduce the number of transfusion related transmissions.

Once a patient is found to have HCV, the patient needs to be counseled to reduce the risk of HCV transmission to others. The physician should also offer counseling on treatment, reducing alcohol usage and immunization with HAV, HBV, pneumococcal and influenza vaccines. HCV negative persons with ongoing risk factors also require counseling and immunization with HAV and HBV vaccines (Backmund et al., 2005 & Edlin et al., 2005).
Treatment currently available and side effects associated

The National Institutes of Health recommends treatment for HCV for those with a positive test result indicating hepatitis C virus circulating in an individual's bloodstream, biopsy indicating significant liver damage and elevated levels of a liver enzyme, alanine aminotransferase (ALT). The standard of care for HCV as of date is weekly injections of a drug called pegylated interferon alfa combined with twice-daily oral doses of ribavirin, a broad-spectrum antiviral agent. Two pegylated interferon medications are available, peginterferon alfa-2b (Peg - Intron) and peginterferon alfa-2a (Pegasys). Combined pegylated interferon and ribavirin clear HCV infection in 40 percent to 80 percent of those treated. Its success often depends on the type of infection. It clears infection in up to half the people with genotype 1 and in up to 80 percent of those with genotypes 2 and 3. If one course of combined pegylated interferon and ribavirin doesn't clear HCV in an individual infected with HCV, a second course of combination therapy is recommended. If the viral load declined during the first round of medications, a second round may clear the virus completely. Even if there was no change in viral load during the first course of treatment, a second course may help reduce the damage.

Interferon side effects include severe flu-like symptoms, irritability, depression, concentration and memory problems, skin irritation, fatigue, and insomnia. Ribavirin can cause a low red blood cell count (anemia), itchiness, nasal congestion, skin irritation, fatigue, and birth defects. Combination therapy including pegylated interferon and ribavirin may cause psychosis or suicidal behavior in a small number of people. For this reason, treatment with interferon is not recommended in those with a history of uncontrolled major depression. It was also not advisable in those who are pregnant or have untreated thyroid disease, low blood cell counts or autoimmune disease, or with the habit of taking alcohol. Side effects from combined pegylated interferon and ribavirin are generally most severe during the first few weeks of treatment, and may be improved with pain relief medications and antidepressants. However, some people taking interferon need their dosage reduced because of severe side effects and others must stop treatment.
Possible source of alternatives

No complementary medicine or alternative medicine to allopathy have been scientifically proved to cure or even ease symptoms of HCV. However, some people are turning to herbs for relief. They use herbs either to help to cure hepatitis or to deal with side effects of interferon. The harmful side effects of modern medicine can include: sudden hearing loss, anemia and other forms of low blood cell counts, headaches, heart, eye, liver, or kidney problems and disorders of the mind, including depression. Among potential herbal therapies for HCV, the most promising alternative treatment seems to be the herb commonly called milk thistle.

Preliminary studies in animals showed that milk thistle may help protect the liver from injury by a variety of toxins and limit the damage from them. To date, the most reliable and also quite preliminary, studies on human show that milk thistle does not cure liver disease but that it may improve the way the liver works in patients with cirrhosis. However there is no current evidence to indicate that milk thistle directly affects HCV.

In Germany, where many herbs are regulated and prescribed as drugs for various diseases, health authorities have approved milk thistle as a complementary treatment for cirrhosis, hepatitis, and similar liver conditions. But a great deal of research still is needed before this alternative therapy could be considered a standard treatment option in the United States.

Plant as a cure for liver disease

Herbal therapy has been an important part of health and wellness for hundreds of years. Herbs contain many substances that are good for the body and are therefore used in the treatment of various illnesses. Along with traditional medicine, herbs can be used to help the treatment of a disorder. Recent surveys have indicated that Americans now make more visits to healthcare professionals who specialize in alternative medicine than to doctors who practice conventional medicine. (Alternative medicine is any therapy used to treat an illness that is not within the realm of conventional and/or accepted medical therapies.) People with liver disease are no exception to this trend. Dozens of publications and books proclaim the proficiency of herbs for the treatment of medical conditions including hepatitis, cirrhosis, and other liver diseases. The use of
traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (UNESCO, 1996). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). Moreover, in these societies, herbal remedies have become more popular in the treatment of minor ailments, and also on account of the increasing costs of personal health maintenance. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today, face either extinction or loss of genetic diversity. Research recently published in the World Journal of Gastroenterology (volume 13, issue 48) shows the aqueous leaf extract (ALE) of A. ilicifolius—a plant that grows in India, prevents cancer-related DNA alterations and chromosomal damage in mice specifically bred to develop liver tumors. In addition, the animals lived far longer than they were expected to survive. Use of plants as a source of medicine has been inherited and is an important component of the health care system in India.

In India, traditional systems have remained quite separate from Western medicine. In addition to Ayurvedic medicine, which has a Hindu origin, Unani medicine with its Muslim and Greek roots is another widely practiced herbal tradition in India. The renewed interest in medicinal plants has focused on herbal cures among indigenous populations around the world, especially those in the tropical rain forests. It is hoped that these investigations will add new medicinal plants to the world’s pharmacopoeia before they are lost forever. In addition to the destruction of the forests, the erosion of tribal cultures is also a threat to herbal practices.
## Plants used against liver diseases (Kotoky and Das, 2008)

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Local names</th>
<th>Parts used</th>
<th>Mode of use</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alocasia indica</em></td>
<td>Man kachu</td>
<td>Rhizome</td>
<td>Rhizomes paste with molasses as pills of 5 - 10 g daily twice daily for 8 - 15 days.</td>
</tr>
<tr>
<td><em>Adhatoda vasica</em> Nees</td>
<td>Baga-bahok</td>
<td>Leaves</td>
<td>Fresh aerial part (5 - 10 g) juice with honey thrice daily for 2 - 3 weeks.</td>
</tr>
<tr>
<td><em>Ananas comosus</em></td>
<td>Mati-kothal</td>
<td>Fruit juice concentrate</td>
<td>Fresh juice of fruit (50 g) with a little salt to taste orally 2 - 3 times daily 3 - 4 weeks.</td>
</tr>
<tr>
<td><em>Boerhaavia diffusa</em></td>
<td>Punannava</td>
<td>Arial part</td>
<td>Fresh aerial part (5 - 10 g) juice concentrates thrice daily for 2 - 3 weeks.</td>
</tr>
<tr>
<td><em>Carica papaya</em></td>
<td>Omita</td>
<td>Fruits/Seeds</td>
<td>Fruit/Seeds (sometimes) juice concentrate, 10 ml with minimum salt to taste and Piper (2 g) thrice daily for 2 - 3 weeks.</td>
</tr>
<tr>
<td><em>Cajanas cajan</em></td>
<td>Rahar</td>
<td>Tender leaves</td>
<td>Tender leaves fresh juice (5 - 10 g) given orally with a little salt to taste for 7 - 15 days.</td>
</tr>
<tr>
<td><em>Cessampelos pareira</em></td>
<td>Saru thupukilata</td>
<td>Arial part</td>
<td>Fresh Plant juice (5 - 10 g) along with Piper powder (1 g) orally thrice daily for 5 - 10 days.</td>
</tr>
<tr>
<td><em>Eclipta alba</em></td>
<td>Keharaj</td>
<td>Leaves</td>
<td>Fresh leaves juice (5 - 10 g) along with black pepper powder (1 g) with salt to taste given orally 2 - 3 times for 2 - 3 weeks.</td>
</tr>
<tr>
<td><em>Ficus religiosa</em></td>
<td>Ahat</td>
<td>Stem bark of southern side</td>
<td>Fresh bark juice (5 - 10 g) along with black pepper powder (1 g) and given orally 2 - 3 times for 2 - 3 weeks.</td>
</tr>
<tr>
<td><em>Gardenia jasminoides</em></td>
<td>Tagar</td>
<td>Leaves</td>
<td>Leaves/flower juice concentrate, 5 ml with minimum salt thrice daily for 2 - 3 weeks.</td>
</tr>
<tr>
<td><em>Glycosmis pentaphylla</em></td>
<td>Saoul Dhowa</td>
<td>Tender leaves and Stem bark</td>
<td>A paste prepared (3 - 5 g) of tender leaves (10 g) given orally daily for 2 - 3 times for 2 - 3 weeks.</td>
</tr>
<tr>
<td>Name of the plant</td>
<td>Local names</td>
<td>Parts used</td>
<td>Mode of use</td>
</tr>
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</tr>
<tr>
<td><em>Leucas lavendulaefolia</em></td>
<td>Dron</td>
<td>Leaves</td>
<td>Shade dried powdered leaves decoction with molasses thrice daily for 10 - 15 days/many people use fresh tender leaf - juice with honey to taste.</td>
</tr>
<tr>
<td><em>Mentha arvensis</em></td>
<td>Podina</td>
<td>Leaves</td>
<td>Fresh juice with marc (10 g) with a little salt to taste twice daily for 2 - 3 days</td>
</tr>
<tr>
<td><em>Phyllanthus niruri</em></td>
<td>Bhui - amlakhi</td>
<td>Seed and leaves</td>
<td>Whole plant powder (5 g) given orally twice a day for 15 - 25 days.</td>
</tr>
<tr>
<td><em>Punica granatum</em></td>
<td>Dalim</td>
<td>Leaves and seeds</td>
<td>Powder of leaves and seeds (5 - 10 g) separately given for 3 - 4 weeks.</td>
</tr>
<tr>
<td><em>Sida cordifolia</em></td>
<td>Sonbarial</td>
<td>Root</td>
<td>Decoction of fresh roots (50 g) along with Piper powder (2 g) given orally twice daily for 2 - 3 weeks.</td>
</tr>
<tr>
<td><em>Swertia chirata</em></td>
<td>Chirota</td>
<td>Leaves</td>
<td>Overnight cold extract of the leaves with honey to taste given orally for 5 - 10 days.</td>
</tr>
<tr>
<td><em>Saccharum officinarum</em></td>
<td>Kuhiar</td>
<td>Stem</td>
<td>Juice Concentrate of stem (10 ml) 3 times orally for 3 - 4 weeks.</td>
</tr>
<tr>
<td><em>Stephania hernandifolia</em></td>
<td>Borthupukilota</td>
<td>Whole arial part</td>
<td>Whole arial part fresh juice (5 - 10 g) given orally with a little salt to taste for 7 - 15 days.</td>
</tr>
<tr>
<td><em>Terminalia chebula</em></td>
<td>Silikha</td>
<td>Seeds</td>
<td>Powder of 5 - 6 fruits given orally with honey thrice daily for 7 - 10 days.</td>
</tr>
<tr>
<td><em>Vitex negundo</em></td>
<td>Pochatia</td>
<td>Leaves</td>
<td>Fresh leaf juice with a little sugar to taste orally 2 - 3 times daily 7 - 15 days.</td>
</tr>
</tbody>
</table>
Details of Medicinal plants that showed antiviral activity

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Medicinal plant used</th>
<th>Virus</th>
<th>Antiviral effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Azadirachta indica</em>&lt;br&gt;Juss. (Neem)</td>
<td>Dengue virus type-2</td>
<td>The aqueous extract of neem leaves inhibited DEN-2 both <em>in-vitro</em> and <em>in-vivo</em></td>
<td>Parida <em>et al.</em>, (2002)</td>
</tr>
<tr>
<td></td>
<td><em>Boehmeria nivea L.</em>,</td>
<td></td>
<td>A root extract of <em>Boehmeria nivea</em> reduced HBV production in an <em>in-vitro</em> and <em>in-vivo</em> model</td>
<td>Huang <em>et al.</em>, (2006)</td>
</tr>
<tr>
<td></td>
<td><em>Ganoderma lucidum</em>&lt;br&gt;<em>Sophorae flavescentis</em></td>
<td>Hepatitis B virus</td>
<td>Liquid fermentation broth showed antiviral activity.</td>
<td>Li <em>et al.</em>, (2005)</td>
</tr>
<tr>
<td></td>
<td><em>Polygononum cuspidatum</em>&lt;br&gt;Sieb. &amp; Zucc.</td>
<td></td>
<td>Inhibits hepatitis B virus in a stable HBV-producing cell line</td>
<td>Chang <em>et al.</em>, (2005)</td>
</tr>
<tr>
<td></td>
<td><em>Oenanthe javanica</em></td>
<td></td>
<td>Inhibitor of HBsAg and HBeAg secretion</td>
<td>Wang <em>et al.</em>, (2005)</td>
</tr>
<tr>
<td></td>
<td><em>P. amarus L.</em>,</td>
<td></td>
<td>Inhibitory effect on HBV polymerase activity</td>
<td>Ott <em>et al.</em>, (1997)</td>
</tr>
<tr>
<td></td>
<td><em>Saxifraga melanocentra</em></td>
<td></td>
<td>A compound namely 1,2,3,4,6-penta-O-galloyl-beta-d-glucoside isolated from <em>Saxifraga melanocentra</em></td>
<td>Zuo <em>et al.</em>, (2005)</td>
</tr>
<tr>
<td>S.No.</td>
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<td>Virus</td>
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<td>Reference</td>
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</tr>
<tr>
<td>4</td>
<td><em>Carissa edulis</em></td>
<td>Herpes simplex virus (HSV)</td>
<td>1346TOGDG and geraniin isolated from <em>Phyllanthus urinaria</em> inhibited HSV-1 and HSV-2, respectively</td>
<td>Yang <em>et al.</em>, (2007)</td>
</tr>
<tr>
<td></td>
<td><em>Podophyllum peltatum</em></td>
<td></td>
<td>A medicinal plant exhibiting strong anti-HSV 1, and 2 activities both <em>in-vitro</em> and <em>in-vivo</em></td>
<td>Tolo <em>et al.</em>, (2007)</td>
</tr>
<tr>
<td>5</td>
<td>Black soybean extract</td>
<td>Human adenovirus type 1</td>
<td>Inhibition of human adenovirus type 1 and coxsackievirus B1 in a dose-dependent manner</td>
<td>Yamai <em>et al.</em>, (2003)</td>
</tr>
<tr>
<td>6</td>
<td><em>Phyllanthus amarus</em></td>
<td>Human immunodeficiency virus</td>
<td>Inhibits HIV replication both <em>in-vitro</em> and <em>in-vivo</em></td>
<td>Notka <em>et al.</em>, (2004)</td>
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<td></td>
<td>Olive leaf extract</td>
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<td>Inhibits acute infection and cell-to-cell transmission of HIV-1</td>
<td>Lee-Huang <em>et al.</em>, (2003)</td>
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<td>7</td>
<td><em>Geranium sanguineum L.</em></td>
<td>Influenza virus</td>
<td>A medicinal plant reducing the infectivity of various influenza virus strains <em>in-vitro</em> and <em>in-vivo</em></td>
<td>(Pantev <em>et al.</em>, (2007)</td>
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<td>Elderberry extract</td>
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<td>A randomized, double-blinded placebo-controlled study revealed that elderberry extract seems to offer an efficient, safe and cost-effective treatment for influenza</td>
<td>Zakay-Rones <em>et al.</em>, (2004)</td>
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<td>8</td>
<td>Guazuma ulmifolia Lam.</td>
<td>Poliovirus</td>
<td>Both plants extract inhibited poliovirus replication, as well as, blocked the synthesis of viral antigens in infected cell cultures</td>
<td>Felipe et al., (2006)</td>
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<td>9</td>
<td>Lycoris radiate</td>
<td>SARS</td>
<td>Lycorine, isolated from Lycoris radiate possesses anti-SARS-CoV</td>
<td>Li et al., (2005)</td>
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<td>Olea europaea L.</td>
<td>Viral haemorrhagic septicaemia virus (VHSV)</td>
<td>Leaf extract inhibited viral replication</td>
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**Active principles in plants**

The active principles in medicinal plants are chemical compounds known as secondary plant products. Some secondary products discourage herbivores, others inhibit bacterial or fungal pathogens. Two major categories of these compounds are alkaloids and glycosides. More than 3000 alkaloids have been identified in 4000 plant species; most occur in herbaceous dicots and also in fungi. Alkaloids contain nitrogen and are usually alkaline (basic), and they have a bitter taste. Their most pronounced actions are on the nervous system, where they can produce physiological and/or psychological results. The difference between a medicinal and a toxic effect of many alkaloids is often a matter of dosage. Glycosides are so named because a sugar
molecule (glyco-) is attached to the active component. Glycosides are generally categorized by the nature of the nonsugar or active component.

**Plants used to cure liver disease**

Botanical medicines have been used traditionally by herbalists and indigenous healers worldwide for prevention and treatment of liver disease. Clinical research in this century has confirmed the efficacy of several plants in the treatment of liver disease, while basic scientific research has uncovered the mechanisms by which some plants provide their therapeutic effects.

The most important classes of herbs for functional liver disease are cholagogues (increase bile flow), choleretics (increasing bile production) and carminatives (expelling gas and antispasmodic). Cholagogue plants usually have a bitter flavor, and all bitter plants are cholagogic to some degree. Some important traditional cholagogues are dandelion (*Taraxacum officinale*), greater celandine (*Chelidonium majus*), and wormwood (*Artemesia absinthum*). Use of cholagogues in functional liver and gall bladder disease only after full examination has ruled out organic disease. They are not used during an acute attack of gall stones, gall bladder pain, or other abdominal inflammation. There is some controversy about whether cholagogues may promote a crisis in a patient with gall stones, i.e. whether stimulation of bile may cause a stone to move into the ducts, blocking them. Wormwood or celandine as a tea or tincture for 2 - 3 weeks, or take dandelion in any form for 4 - 6 weeks can be taken as a cure. Dandelion may be taken in a tea or tincture, or even better. The leaves can be taken in salads, cooked as greens, or juiced (Paul Bergner, 1997).

To cure liver ailments, tribals collect rhizomes of *Acorus calamus Linn* which is dried and powdered and consumed with water. When patients are suffering from jaundice, the leaves of beetle vine and of *Andrographis paniculata* are given to the patients to chew for few days. In case of acute jaundice, patients are asked to chew daily 4 - 5 fresh leaves of *Phyllanthus niruri* for 20 - 25 days. The same has been found to be very useful in case of severe jaundice but the patients are asked to chew 4 - 5 leaves twice a day for 40 - 45 days. The seeds of *Cassia tora Linn* and leaves of *Azadirachta indica* are also chewed to cure liver ailments. Paste of *Cuscuta reflexa* is
Liver cancer is the fifth most common cancer in the world with a poor prognosis. About three quarters of the cases of liver cancer are found in Southeast Asia, including China, Hong Kong, Taiwan, Korea, India and Japan. The frequency of liver cancer in Southeast Asia and sub-Saharan Africa is greater than 20 cases per 100,000 population. Moreover, recent data show the frequency of liver cancer in the U.S. overall is rising. With the increasing trend in the incidence of cancers in India, biomedical research directed at early detection and diagnosis, prognosis and survival, as well as prevention of progression of malignancy, is of prime importance. A research team from Jadavpur University investigated the primary chemopreventive mechanisms of Acanthus ilicifolius. Acanthus ilicifolius is distributed widely throughout the mangroves of India, including Sunderbans in West Bengal and the Andamans. The results showed the aqueous leaf extract of the plant was substantially effective in preventing hepatic DNA alterations and sister-chromatid exchanges in tumor-bearing mice. This research opens up a promising avenue in cancer chemoprevention with the use of indigenous plants (Chakraborty et al., 2007).

Curcuma longa is a member of the ginger family. It is a tropical plant extensively cultivated in the tropical areas of Asia, and to a lesser extent in Africa. It is the source of the spice turmeric, which is derived from the dried, ground rhizome. Traditional applications include the treatment of gastrointestinal colic, flatulence, hemorrhage, hematuria, menstrual difficulties and jaundice (Leung, 2006). Green, black, and oolong teas all derive from the leaves of Camellia sinensis, which is cultivated widely in India, China, Japan and Indonesia. Green tea has been found to provide protection to the liver against a variety of toxic insults, including the industrial solvent 2-nitropropane which is also found in cigarette smoke (Sai et al., 1998).

Picrorhiza kurroa, a known hepatoprotective plant, was studied in experimental and clinical situations and it has been found to be helpful in clinical and biochemical recovery in acute hepatitis. Jayaram found Picrorhiza kurroa to be effective in HBeAg seroconversion in 28.5% of patients compared to 16% seroconversion with placebo (Jayaram, 1992).
Plants chosen for this study

A literature survey was carried out in detail before the selection of medicinal plants for screening anti HCV activity. Based on the ethnobotanical data and the information collected from siddha/ayurvedic practitioners three plants were chosen for this study. The selected plants were Boerhavia diffusa, Eclipta alba and Phyllanthus amarus.

Boerhavia diffusa

Boerhavia diffusa, commonly known as punarnava in Sanskrit is a herbaceous plant. The whole plant or its specific parts like leaves, stem and roots are known to have medicinal properties and have a long history of use by tribal people in India (Dhar et al., 1968). The major active principle present in the root is alkaloidal and is known as punarnavine. In the traditional system of medicine, B. diffusa roots have been widely used for the treatment of dyspepsia, jaundice, enlargement of spleen, abdominal pain (Kirtikar and Basu, 1956) and as an antistress agent. The worldwide use of B. diffusa roots to treat liver disorders was validated when researchers demonstrated in 1980 and 1991 that its root extract had antihepatotoxic properties (Rawat et al., 1997 & Chandan et al., 1991).

Taxonomy

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PLATE - 1

Boerhavia diffusa
Vernacular Names

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<td>Punarnava</td>
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Origin and distribution

*B. diffusa* is indigenous to India, it is found throughout the warmer parts of the country up to an altitude of 2000 m in the Himalayan region. It grows well on wastelands and in fields after the rainy season (Chopra, 1969). The plant is also cultivated to some extent in West Bengal (CSIR, 1988). The genus *Boerhavia* has several species, and is distributed in the tropical, subtropical and temperate regions of the world (Heywood, 1978). It is found in Australia, China, Egypt, Pakistan, Sudan, Sri Lanka, South Africa, USA and in several countries of the Middle East. Out of the 40 species of this genus, 6 species are found in India Viz. *B. diffusa*, *B. chinensis*, *B. erecta*, *B. repens*, *B. rependa* and *B. rubicunda* (CSIR, 1988 & Chopra, 1969).

Biology

*B. diffusa* is a perennial creeping weed, prostrate or ascending herb, up to 1 m long or more, having spreading branches. Grows as common weed. The roots are stout and fusiform with a woody root stock. Cortex of the root composed of starch grains and layer of thin walled, oval to polygonal parenchymatous cells. Vessels having simple pits and reticulate thickening, fibres aseptate, thick walled, spindle shaped with pointed ends. Numerous raphids of calcium oxalate in single or in groups present in cortical region (Prasad, 1948). The stem is prostrate, woody or succulent, epidermal layer containing multicellular, uniseriate glandular trichomes consisting of ellipsoidal or clavate head and a stalk of 8 - 12 cells, cortex consists of 1 - 2 layers of collenchymas and 6 - 7 layers of parenchyma. Endodermis indistinct. Stele composed of two large vascular bundles in the centre, surrounded by a ring of 6 - 12 loosely arranged bundles, intrafascicular cambium present. The shape of the leaves varies considerably from ovate.
- oblong, round or subcordate at the base and smooth above. Margins of the leaves are smooth, wavy or undulate. Both the upper and lower epidermis show the presence of numerous multicellular glandular hairs and anomocytic stomata. Palisade one layered, spongy parenchyma 2 - 4 layered, cells polyhedral or isodiametric in shape, with distinct intercellular spaces (Datta and Mukerji, 1952). The upper surface of the leaves is green, smooth and glabrous, whereas it is pinkish white and hairy beneath. Flowers are minute, subcapitate, present 4–10 together in small bracteolate umbels, forming axillary and terminal panicles. Fruits highly viscid, easily detachable one-seeded < 3.5 mm, club-shaped, ridges 4 - 5, wings 0. Mode of propagation is by seeds.

Floral Formula

\[ \text{Br, Br1, O, (++) P5, A(5), G(2)} \]

Ethnobotanical uses

*Boerhavia* roots are used in the treatment of piles by the inhabitants of the Garhwal Himalaya (Uttaranchal). Its leaves are cooked and eaten in Assam, where it is commonly found in the markets. In Purulia (West Bengal), tribals eat this plant as vegetable. The root paste is used to cure bloody dysentery by the Bhils of the Jhabua district in Madhya Pradesh. *B. diffusa* is used for curing ailments such as leukorrhrea, rheumatism, and stomach ache by the Sahariya tribe in the Lalitpur district of Uttar Pradesh. The decoction of the plant is given in the treatment of nodules in the body. The root juice is used in treating asthma, scanty urine, and internal inflammation disorders. This plant is also used by the tribes of Ambikapur district, Madhya Pradesh for the treatment of elephantiasis. In the Indo-Nepal Himalayan terai region, the tribals harvest this plant for medicinal purposes, mainly for flushing out the renal system, to treat seminal weakness and blood pressure (Mitra and Gupta, 1997).

Chemical constituents

(Mishra and Tiwari, 1971), punarnavoside (Jain and Khanna, 1989), liirodendrin (Aftab et al., 1996) and a glycoprotein having a molecular weight of 16–20 kDa (Verma et al., 1979) have been isolated and studied in detail for their biological activity. Chopra et al. (1923), reported that the plant contained large quantities of potassium nitrate, besides punarnavine. The herb and roots are rich in proteins and fats. The herb contains 15 amino acids, including 6 essential amino acids while the root contains 14 amino acids including 7 essential amino acids. Seth et al., (1986), isolated a new antifibrinolytic compound ‘punarnavoside’ from the roots of B. diffusa. Phytochemical screening of the roots from garden-grown in-vivo plants of B. diffusa of different ages revealed that the maximum alkaloid content (2%) accumulated in the roots of 3-years old mature plants.

Pharmacology

Pharmacological studies have demonstrated that punarnava possesses punarnavoside, which exhibits a wide range of properties-diuretic (Gaitonde et al., 1974), antiinflammatory (Bhalla et al., 1968), antifibrinolytic (Jain and Khanna, 1989), anticonvulsant (Adesina, 1979), antibacterial (Olukoya et al., 1993), antistress agent, antihepatotoxic (Mishra, 1980, Chandan et al., 1991 and Rawat et al., 1997), anthelmintic, febrifuge, antileprosy, antiasthmatic, antiscabies and anti urethritis (Nadkarni, 1976) and antinematodal activity (Vijayalakshmi et al., 1979). An aqueous extract of thinner roots of B. diffusa at a dose of 2 ml kg-1 exhibited marked protection of various enzymes such as serum glutanicoxaloacetic transaminase, serum glutanic-pyruvic transaminase and bilirubin in serum against hepatic injury in rats (Rawat et al., 1997).

Singh and Udupa (1972) reported that dried root powder showed curative efficiency when administered orally for one month to children or adults suffering from helminth infection. The subjects became worm-free within five days of treatment. The drug singly or in combination with other drugs was found to be effective in liver disorders, heart diseases, respiratory tract infections, leukorrhea, spermatorrhea, etc. The purified glycoprotein from B. diffusa exhibited strong antimicrobial activity against many bacteria (Awasthi and Menzel, 1986). With much of the clinical research validating bacteriophages long history of different uses in natural medicine the commercial bulk of punarnava in India represents heterogeneous medicinal uses. The roots of B. diffusa are a rich source of a basic protein which is used for inducing

**Eclipta alba**

*Eclipta alba* grows commonly in moist places as a weed all over the world. It is widely distributed throughout India, China, Thailand and Brazil. *Eclipta alba* (L.) Hassk. (syn. *Eclipta prostrata* L.), commonly known as False Daisy, yerba de tago and bhringraj is a plant belonging to the family Asteraceae. In Ayurveda a large number of indigenous drugs have been mentioned possessing analgesic properties. The total ethanol extract of *E. alba* have been shown to possess analgesic properties (Sawant et al., 2004).

**Taxonomy**

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**Vernacular name**

| Tamil           | Kayanthakora, Karippan, Karisalangkani and Karisalai |
| Hindi           | Bhangra                                                 |
| Kannada         | Garagadasoppu                                           |
| Malayalam       | Kyonni                                                  |
| Sanskrit        | Ajagara                                                 |
| Telugu          | Guntagalijeru                                           |
| Bengali         | Keshori                                                 |
| Marathi         | Maka                                                    |

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PLATE - 2

_Eclipta alba_
Origin and distribution

*Eclipta alba* is native to the tropical and subtropical regions of the world, usually found on poorly drained wet areas, along streams, ditches in marshes and on the dikes of rice paddies. However, it is also common in lawns and in upland conditions where rainfall is about 1,200 mm or more. It can grow under wet, saline conditions but is often a weed of drier sites in plantation crop (Holm *et al.*, 1977). It is a weed in moist bottomlands and muddy places (Wiggins and Porter, 1971). In Guam, a pantropical weed of open, sunny and wet localities (Stone Benjamin, 1970). In Hawaii it is naturalized in disturbed areas (Wagner *et al.*, 1999). In Fiji it is found near sea level and said to be locally common as a naturalized weed in waste places, cultivated areas and open fields often near ditches (Smith, 1991). In New Guinea, it is a common plant of wet situations, foreshores, streamsides, roadside ditches and a weed of wet pasture. It is also found at low altitudes (Henty and Pritchard, 1975).

Biology

This tropical annual plant is a creeping and moisture-loving herb. It is usually found on poorly drained, wet areas; along streams and ditches in marshes; and on the dikes of rice paddies. However, it is also common in lawns and in upland conditions where rainfall is about 1,200 mm or more. It can grow under wet, saline conditions but is often a weed of drier sites in plantation crops. Root well developed, cylindrical, greyish. The roots are emetic and purgative. Stems and branches are strigose and hairy. Leaves lanceolate, elliptic or oblong, acute-acuminate, acute at base, subsessile, subtrinerved, entire or serrulate, pilose, mostly 2 - 10 cm long and 1 - 4 cm wide, heads terminal and axillary, all tuberculate, black, glabrous except for a few apical hairs, depressed - truncate at apex, with 1 - 3 minute marginal teeth, about 2.8 mm long, marginally ribbed (Stone Benjamin, 1970). Floral heads 6 - 8mm in diameter, solitary, white, achene compressed and narrowly winged. Ray flowers are fertile, pistillate. Disk flowers are white. Lobes acute, 3mm long. Stamens 4. Anthers purple, 5 - .6mm long, included and connate around style. Style whitish at apex. Achenes 2.5mm long. Pappus absent to a minute crown. Receptacle flat. Chaff 3mm long, translucent yellow and antornosely barbellate.

Floral formula

\[ Br, Brl, O, %, K_a, C(5), A(5), G(2) \]
Ethnobotanical Use

Plant is bitter, hot, sharp, dry in taste and is used in ayurveda for the treatment of Kapha and Vata imbalances. In India, the plant is known as bhangra, "bhringaraj" or bhringraja. An other plant Widelia calendulacea is also known by the same name, but Eclipta has white flowers so called white bhangra and Widelia has yellow flower so it is called yellow Bhangra (Puri, 2003). The expressed leaf juice is applied along with honey is a popular remedy for catarrh in infants. A preparation obtained from the leaf juice boiled with sesame or coconut oil is used to render the hair black and luxuriant. An oil prepared with amla, bhringraj and sometimes with brahmi is well known in India as Amla Bhringraj oil, which is said to blacken the hair. Plant is rubbed on the gums in toothache and applied with a little oil for relieving headache and with sesame oil in elephantiasis. Roots of Eclipta alba are emetic and purgative.

In Taiwan, entire plant is used as a remedy for the treatment of bleeding, haemoptysis, haematuria and itching, hepatitis, diphtheria and diarrhoea. In China, as a cooling and restorative herb, which supports the mind, nerves, liver and eyes. The leaf extract is considered to be powerful liver tonic, rejuvenative, and especially good for the hair. A black dye obtained from Eclipta alba is used for dyeing hair and tattooing. Eclipta alba also has traditional external uses, like athlete foot, eczema and dermatitis, on the scalp to address hair loss and the leaves have been used in the treatment of scorpion strings. It is used as anti venom against snakebite in China and Brazil. In Ayurveda the plant is considered a rasayana for longevity and rejuvenation. Recent studies have shown that it has a profound antihepatotoxic activity. A cardiodepressant activity was also observed in it when used for hepatic congestion. A complete symptomatic relief in epigastric pain, nausea and vomiting in ulcer patients has also been observed (Puri, 2003).

The roots have emetic and purgative properties and it has been applied externally as an antiseptic to ulcers and wounds in cattle. Since the harvest of medicinal plants on a large scale from their natural habitats is leading to a depletion of plant resources, the conservation of these valuable genotypes is imperative. Micropropagation via shoot culture, often utilized to maintain clonal fidelity, would be a appropriate in this respect (Sen and Sharma, 1991). Large scale, unrestricted exploitation of this natural resource to meet the ever increasing demand for it by the
Indian pharmaceutical industry coupled with limited cultivation and insufficient attempts for its replenishment, this medicinally important and endangered plant species have markedly depleted (Sharma and Kumar, 1998, Sing, 1998 and Pandey et al., 1993). In recent years, there has been an increased interest in in-vitro culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants (Prakash et al., 1999, Tejavathi and Shailaja, 1999, Ajithkumar and Seeni, 1998 and Sahoo and Chand, 1998). Tissue culture techniques can play an important role in the clonal propagation of elite clones and germplasm conservation of this medicinal herb *Eclipta alba*. There have been few reports to date on micropropagation in the genus using nodal explants (Borthakur et al., 2000 & Franca et al., 1995). However, the establishment of a micropropagation protocol for *E. alba* constitutes an useful tool for large scale plant production, assuring continuous availability of plant material appropriate for the study of factors that influence the production of the secondary metabolites as well as for strategies of in-vitro culture to increase the yield of these active principles accumulated in cultures of *E. alba*.

**Chemical constituents**

Das and Chakravarty (1991), isolated ecliptal, a new terthienyl aldehyde from the plant. The leaves of the plant contain stigmasterol, a-terthienyle methanol, 2-formyl-terthienyl, wedelolactone, de-me-wedelocactone and 7-O-glucosies. Aerial parts of the plant contain β-amyrin, wedelolactone, luteolin-7-glucoside, phytosterol A and its glucosides (Asolkar et al., 1992).

**Pharmacology**

This species is widely used in traditional Chinese herbal medicine and in Ayurveda (Bown, 1995). It is considered to be the best remedy for the hair and is also used as a rejuvenative and liver tonic (Chevallier, 1996 & Bown, 1995). The whole plant is astringent, deobstruent, depurative, emetic, febrifuge, opthalmic, purgative, styptic and tonic (Yeung, 1985 & Stuart, 2000). It is used internally in the treatment of dropsy and liver complaints (Lassak, 2005) anaemia and diphtheria (Bown, 1995), tinnitus, tooth loss and premature greying of the hair (Yeung, 1985). Externally, it is used as an oil to treat hair loss and is also applied to athlete's foot, eczema, dermatitis and wounds (Chevallier, 1996 & Bown, 1995). The plant juice is used in the treatment
of catarrhal problems and jaundice. The leaves are used in the treatment of scorpion stings. The plant is harvested as it comes into flower and is dried for later use (Bown, 1995). The roots are emetic and purgative (Chopra et al., 1986). They are applied externally as an antiseptic to ulcers and wounds, especially in cattle (Chopra et al., 1986). In ayurvedic medicine, the leaf extract is considered to be a powerful liver tonic, rejuvenative and especially good for the hair (Kritikar and Basu 1975 and Chopra et al., 1955). A black dye obtained from E. alba is used for tattooing. E. alba also has traditional external uses, like athlete foot, eczema and dermatitis, on the scalp to address hair loss and the leaves have been used in the treatment of scorpion stings. It is used as antivenom against snakebite in China and Brazil.

E. alba is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases (Anonymous, 1952). The alcoholic extract of the plant has shown antiviral activity against Ranikhet disease virus (Anonymous, 1952). The plant is commonly used in hair oil all over India for healthy black and long hair. The fresh juice of leaves is used for increasing appetite, improving digestion and as a mild bowel regulator. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma and popularly used to enhance memory and learning. The plant has a reputation as an antiageing agent in Ayurveda. Eclipta alba is used as a general tonic for debility. Externally it is used for inflammation, minor cuts and burns and the fresh leaf juice is considered very effective in stopping bleeding. Leaf juice mixed with honey is also used for children with upper respiratory infections and also used in eye and ear infections. E. alba is a source of coumestan-type compounds used in phytopharmaceutical formulations of medicines prescribed for treatment of cirrhosis of the liver and infectious hepatitis (Murphy et al., 1996). E. alba is widely used in India as a cholagogue and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gall bladder (Orning, et al., 1980). Coumestan-type compounds, wedelolactone and dimethyl wedelolactone, have been isolated as the main active principles of E. alba, both constituents exhibiting antihepatotoxic activity (Franca et al., 1995, Mors et al., 1989 and Wanger et al., 1986). In-vivo tests indicate that wedelolactone neutralizes the lethal and myotoxic activities of rattlesnake venom (Mors et al., 1989). Wedelolactone (WL) and dimethylwedelolactone (DWL) showed potent activity when tested in the trypsin inhibition bioassays (Syed et al., 2003). The shoot extract shows antimicrobial activity
against *Staphylococcus aureus* and *Escherichia coli* (Anonymous, 1952). From the whole plant of *E. alba*, a new triterpene saponin, namely eclalbatin, together with alpha-amyрин, ursolic acid and oleanolic acid have been isolated (Upadhyay *et al.*, 2001).

**Phyllanthus amarus**

*Phyllanthus amarus* is an Indian herb that has been used for more than two thousand years by Ayurvedic practitioners in support of the liver. 'Phyllanthus' means "leaf and flower" because the flower, as well as the fruit, seems to become one with the leaf (Cabieses, 1993).

**Taxonomy**

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<tr>
<td>Hindi</td>
<td>Jamgli aml, Jaramla</td>
</tr>
<tr>
<td>Kannada</td>
<td>Kirunelli</td>
</tr>
<tr>
<td>Malayalam</td>
<td>Kilarnelli, Kilukanelli</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Bhumyamalaki</td>
</tr>
<tr>
<td>Telugu</td>
<td>Nela usirika</td>
</tr>
<tr>
<td>Bengali</td>
<td>Bhuiamla, sadahazurmani</td>
</tr>
<tr>
<td>Marathi</td>
<td>Bhuivali</td>
</tr>
</tbody>
</table>

Some common names of *P. amarus* in North, Central and South America are black catnip, carry-meseed, chanca piedra, djari-bita, egg woman, fini-bita, flor
PLATE - 3

*Phyllanthus amarus*
escondida, gale-of-(the)-wind, hurricane weed, quebra-pedra, quinine creole, quinine weed, seed-under-leaf, stone breaker and yerba de la nina (Morton, 1981).

Origin and distribution

*P. amarus* is widely distributed in all tropical regions of the planet. Paleobotanical studies have not found the exact geographic origin of this plant. This plant may be indigenous to the tropical Americas (Cabieses, 1993, Tirimana, 1987 and Morton, 1981), the Philippines or India (Chevallier, 2000 & Cabieses, 1993).

Biology

*P. amarus* is an annual herb, 10 to 50 cm high. *P. amarus* is a common pantropical weed that grows well in moist, shady and sunny places (Nanden, 1998 & Cabieses, 1993). It is found mainly as a weed in waste lands, agricultural lands and riverbanks. In waste lands it grows abundantly during the rainy season. Stem smooth, cylindrical, 1.5 to 2 mm thick erect stem, naked below and slender. The leaves are alternate, petioles 0.3 to 0.5 mm long, elliptic, oblong or obovate, 3 to 6 mm wide, rounded to slightly pointed at the tip, scarcely oblique on one side at the base, 4 to 12 cm long and about 0.5 cm thick, with 15 to 30 leaves, simple, alternate or opposite and some are leathery. The flowers are alone or usually one male and one (larger) female are in each leaf axil together, very small and diclinous, they cluster in cup-shaped structures, greenish, often with glands. The fruit is a three-lobed capsule extending from the cup and commonly the long stalk pendant (Lewis and Elvin-Lewis, 1977 & Wessels Boer, 1976). The seed capsules on stalks are 1 to 2 mm long, round, smooth, 2 mm wide, with 6 seeds. When the fruits burst open the seeds are hurled away. Seeds are triangular, light brown, 1 mm long, with 5 to 6 ribs on the back (Morton, 1981 & Wessels Boer, 1976).

Floral Formula

\[ Br, Eb, p(5), A 5 G (3) \]

Ethnobotanical uses

The genus *Phyllanthus* has a long history of use in the treatment of liver, kidney and bladder problems, diabetes and intestinal parasites. According to Foo and Wong (1992), in a number of countries, the aerial part of *P. amarus* is highly valued in traditional medicine for its healing properties. In many countries around the world
Plants in the genus *Phyllanthus* are used in folk remedies; therefore this genus is of great importance in traditional medicine (Foo, 1993). Chevallier (2000) reported that *P. amarus* is also used traditionally in India to treat cardiovascular problems. This popular medicinal herb is also a remedy around the world for influenza, dropsy, diabetes and jaundice (Foo, 1993). This plant is traditionally used around the world in the treatment of liver ailments and kidney stones. The Spanish name ‘chanca piedra’ means “stone breaker or shatter stone.” In South America, ‘chanca piedra’ has been used to eliminate gall bladder and kidney stones, and to treat gall bladder infections. *P. amarus* has also shown to work as an antifungal, antibacterial and antiviral agent (Houghton et al., 1996). Foo and Wong (1992), report that in India this plant is used in traditional medicine to treat liver diseases, asthma and bronchial infections. While working with *P. amarus*, in-vitro generated roots have been used for the study. Although roots of the plant were found to have pharmaceutical property (Venkateswaran et al., 1987) like the aerial parts, this plant organ was rarely used for therapeutic evaluation. With the advancement of biotechnology, manyfold production of plant roots within a short time period has become possible and root culture is now a preferred technique for generating a stable source of secondary metabolites (Jasrai and George, 2000, Asolkar et al., 1992).

**Chemical constituents**

The secondary metabolites present in *P. amarus* are alkaloids, flavonoids, hydrolysable tannins, major lignans and polyphenols. Several chemical investigations have been conducted where the structures of most of these phytochemicals were determined by UV, IR, Mass and NMR spectroscopy (Foo, 1993 and Foo, 1992 and Foo and Wong, 1992). Houghton et al., (1996), isolated securinega type alkaloids by Column Chromatography (CC) and preparative Thin Layer Chromatography (TLC).

**Pharmacology**

In Suriname, *P. amarus* is always sold as fresh and dry plant material in the herb markets and the decoctions are used in herbal baths and after labor (Sedoc, 1992, Titjari, 1985 and May, 1982). Heyde (1990), Sedoc (1992) and Nanden (1998), noted that in traditional medicine a herbal decoction is taken to treat bladder and kidney disorders, cramps and uterus complaints. Sedoc (1992), notes that in Suriname a decoction of *P.amarus* is taken along with other herbs to treat stomachache. This plant
decoction works also as an appetizer. *P. amarus* is a restoration herb and can be used as a tonic. According to Heyde (1990), plant extracts of *P. amarus* can be used as blood purifiers, for light malaria fevers and anaemia. *P. amarus* helps to release phlegm (Heyde, 1990) and is used to combat fever, flu (Nanden, 1998) and asthma in combination with other herbs (Titjari, 1985). The plant when boiled with the leaves, is considered to be a diuretic and can be used in treating diabetes, dysentery, hepatitis, menstrual disorders and skin disorders (Heyde, 1990 and Tirimana, 1987). This herb can also be used for constipation (Tjong and Young, 1989). Extracts from the roots can be used for jaundice. Bratati and Datta (1990), report in an evaluation study of *P. amarus* that plant extracts have shown *in-vivo* antifungal, anticancer, antispasmodic and hypoglycaemic activity. According to Thyagarajan *et al.*, (1988), plant extracts from this species have beneficial effects on liver functions. Mehratra *et al.*, (1991) and Unander and Blumberg (1991), showed using *in-vitro* studies that the *P. amarus* extracts (polar fractions) also have antiviral activity and are a potential remedy for HBV infection. A study confirms that *P. amarus* inhibits the activity of the HBsAg (Thyagarajan *et al.*, 1998), HBV DNA polymerase enzyme, suggesting one potential mechanism for antiviral activity (Blumberg *et al.*, 1989). *Phyllanthus amarus* have also been used in traditional medicine practitioners around the world particularly for treatment of jaundice and other liver diseases. It has been proved that *Phyllanthus amarus* encodes various activities against viral hepatitis, hepatoprotective, immunomodulating and anti-inflammatory activities (Thyagarajan *et al.*, 1990, Blumberg *et al.*, 1990). Extensive studies on *Phyllanthus amarus* have confirmed this plant preparation as being anti-viral against hepatitis B and C viruses, hepatoprotective and immunomodulating, as well as possessing anti-inflammatory properties. For the first time in the Indian systems of medicine, a chemo-biological fingerprinting methodology for standardization of *P. amarus* preparation has been patented (Thyagarajan *et al.*, 1993). For the treatment of jaundice and chronic liver diseases unani preparations were approved by the Indian medical practitioner’s co-operative pharmacy and stores (Thyagarajan, 1996). In a clinical trial in acute viral hepatitis (AVH) patients, Jayanthi *et al.*, (1988) used *P. niruri* and compared it with other herbal medicines (Jayanthi *et al.*, 1988). Jayaram and Thyagarajan reported *in-vitro* inhibition of HBsAg secretion by PLC/PRF/5 (Alexander) cell line for 48 h when the cell line was treated with 1 mg/mL concentration of *P. amarus* as a single dose (Jayaram and Thyagarajan, 1996).
Alternative and complementary medicine for Hepatitis C

Interferon is often combined with an antiviral (virus-fighting) drug called ribavirin. Such combination therapies are usually taken for 6 months to 1 year. Approximately 55 percent of patients treated with the combination of interferon and ribavirin for 1 year will achieve a sustained response. Due to the side effects associated with interferon and ribavirin many complementary and alternative medicines are used these days. Some of them includes thymus extract and colloidal silver.

The thymus is a gland that is involved in the regulation of the body's immune response. Products of thymus extract products consist of peptides taken from the thymus glands of cows or calves and are sold as dietary supplements. Often, these products carry claims of boosting immune system functioning to combat diseases, such as hepatitis C. These over-the-counter supplements should not be confused with the prescription drug thymosin alpha-1 (Raymond, 1998).

Silver is a metallic element that is mined as a precious metal. People are exposed to silver, usually in tiny amounts, through their environment, drinking water, food, and possibly work or hobbies. Colloidal silver supplements consist of tiny silver particles suspended in a liquid base. They are often marketed with a variety of unproven health claims, including for immunity, diabetes, cancer, and AIDS (Gulbranson et al., 2000). There are various reasons why people use complementary and alternative medicine for HCV which includes, they have not had a response to initial treatment or to re-treatment with drugs, not willing to have drug treatment or continue it due to side effects or length of treatment, they would like to support their body's fight against damage by HCV and they hear of benefits claimed for some complementary and alternative medicine treatments such as "strengthens the immune system" or "cleanses or rejuvenates the liver" and experiencing problems from other diseases and conditions that can be caused by or worsened by HCV. Because of these reasons, a drug with a drug with out side effects for HCV is the need of the hour.