Discussions
6. Discussion

The liver is an important organ of human body in the sense it is the major metabolic house. It gets inflicted in liver disease patients because of its frequent involvement in systemic infections and malignancies, and its tropism for the HBV and non-A, non-B viruses (Lebovics et al., 1985). Alcohol intake also alters the metabolism of the liver there by the liver gets injured. Inflammation of the liver is known as hepatitis and it may be due to viral or bacterial infection or intake of toxic materials or drug.

A group of viruses have tropism over liver and there by affects the liver. Hepatitis virus infections are an increasing problem, with millions of people all over the world being infected. It is accepted as a significant public health problem with several life altering complications. The mortality rate due to liver disease was 28% in the multiple hepatitis group whereas 6% in HIV mono-infected and 15% and 13% in hepatitis B or C single infections, respectively (De Luca et al., 2002). HCV, an RNA hepatotropic virus, is the leading cause of viral hepatitis worldwide (Poovorawan et al., 2002). The mechanisms of HCV persistence are currently unknown, although it is known that HCV chronicity develops despite humoral and cellular responses to HCV proteins. HCV RNA also shows significant genetic variability (Brechot, 1996). Infection with this virus causes a repertoire of liver diseases in addition to a number of extra-hepatic manifestations such as lichen planus and oral cancer (Poduri, 2003).

In this present study the prevalence of HCV in liver disease patients is 5.6%. The probable reason could be drug addiction, alcoholism, blood transfusion and consumption of blood and blood products. It is interesting to note that none of the control samples tested in this study was found to be positive for HCV. As most of the control samples were from educational institutions there is a possibility of awareness about HCV and its consequences. HCV infection is a major problem in India, where it has been estimated that more than 20 million people are already infected (Khaja et al., 2002). Studies carried out in India by Chowdhury et al., (2003) and Panigrahi et al., (1997) showed lesser prevalence of HCV than that observed in this study.
None of the acute liver disease patients of this study found to have HCV infection. Generally acute liver disease cases will have water borne viral infections like HAV and HEV. As HCV happens to be the blood borne virus there is no wonder to have acute liver disease with out HCV infection. A significant proportion of acute viral hepatitis occurring in India is by an enterically transmitted viral agent (Khuroo, 1980). This also proves the fact that HCV infection is often subclinical and leads to chronic infection in 60-80% of those acutely infected. 6.4% of chronic liver disease patients were found to have HCV in the present study. Whereas population based studies conducted among Mexican adults showed 2% HCV positivity in chronic liver disease (Mendez-Sanchez et al., 2005) which is less than the results of this study.

Most of the HCV positive patients of this study had liver cirrhosis (9.4%). The liver is made up of soft tissue and hence prone to get infection followed by inflammation. There is a possibility of further damage of tissue due to coinfection or super infection or multiple viral infection. The reasons accounted for liver cirrhosis is multi variable hence it is not easy to predict HCV infection is because of liver cirrhosis or liver cirrhosis is because of HCV infection. Consumption of alcohol and hepatotoxic drugs could be the probable reason for liver cirrhosis. Comparatively low incidence (4%) of HCV associated mortality was accounted in a study conducted by Sangiovanni et al., (2006), and is higher in those with other liver related morbidities like alcohol abuse, HBV coinfection, and iron overload due to genetic hemochromatosis.

Male gender was highly infected with HCV than female in this study. This may be due to the fact that mostly male in the subcontinent are involved in intravenous drug use and higher consumption of alcoholic drinks. This concords previous report that male had a higher risk of getting infection than female (Padmapriyadarsini et al., 2006, Guadagnino et al., 1997, Chiaramonte et al., 1996).

Age wise prevalence of HCV in this study was found to be high (10.7%) in those above 60 years of age but the prevalence of HCV between 11-20 years of age is 3.8% which is comparatively low. Though the incidence of HCV is high among the elderly population of this study, it is lower than that reported earlier by Alberti et al., (2002), Di Stefano et al., (2002), Baldo et al., (2000) and Bellentani et al., (1994).
In the present study all HCV positive patients showed elevated levels of all liver enzymes and pigment tested and all of them were chronic liver disease patients with or without cirrhosis. Comparatively no one among the control had elevated LFT. Surprisingly 2 patients of this study group with HCV infection showed normal ALT. Montella et al., (2003) also reported similar type of results.

Genotypic prevalence of HCV in this study showed that genotype 3 was the prevalent one followed by genotype 2 and genotype 1. The nomenclature for banding patterns produced by two sets of double digest (Rsa I/Hae III & Mavl/HinfI) were compared with those described by McOmish et al., (1994) and most of the patterns were found in common for type 1, 2 and 3. The banding pattern of Rsa I/Hae III double digestion of some samples were larger than the expected banding pattern. This may be because of loss of conserved Rsa I site in 5' non coding region by A → T or A → C change and hence it was not possible to distinguish between the genotypes. Similar or such kind of results i.e. anomalous restriction length polymorphism banding patterns have been reported earlier by Dusheiko et al., (1994).

Raghuraman et al., (2003) have reported that genotypes 3, 1 and 4 were prevalent in India. Recently Das et al., (2002) in India reported the predominance of genotype 3 from the study on 153 HCV strains by Inno-Lipa method was relevant to the report of this study done by using RFLP.

With regard to genotyping vs clinical conditions this study showed the preponderance of HCV genotypes 3 and 1 in patients with chronic liver disease. Similar studies in chronic persistent patients exclusively from Northern and Southern India have reported the predominance of either genotype 3 or 1 (Das et al., 2002).

In a study conducted among Southern Indian individuals with diagnosis of non-A, non-B hepatitis revealed the predominance of genotype 1 (87.5%) over genotype 3 (12.5%) by the DNA sequencing method (Valliammai et al., 1995). The global distribution pattern of HCV genotypes has shown 1a and 1b to be prevalent in the
United States (Lau et al., 1995). Similar to the report of this study Genotypes 1, 2 and 3 were seen in Europe (Schroter et al., 2002) and Australia (McOmish et al., 1994).

Coinfection or super infection of other blood borne viruses like HBV, HDV and HIV along with HCV in liver disease patients were found in this study. 1.5% cases of this study were found to have HCV and HBV infection together. HCV and HBV coinfection was found to be high in chronic liver disease patients of this study. With regard to age, HCV and HBV coinfection was found to be high in those above 61 years of age. The probable reason could be age, blood transfusion, iatrogenic or nosocomial infection, immune status of the individual etc. But a study conducted by Gupta et al., (1957) showed maximum coinfection of HCV and HBV among 31-40 years of age. Patients with HBV and HCV coinfection and cirrhosis are a subset of patients at a very high risk for HCC (El-Serag, 2002, Seeff, 2002 and Donato et al., 1998).

Elevated level of ALT and AST was observed in the patients who were coinfected with HCV and HBV. The elevation of ALT and AST is usually related to structural damage to the hepatocytes or this may reflect an improper function of hepatocytes. Similar results were reported by Wojcicki et al., (2002).

When compared to HCV and HBV coinfection, HCV and HIV coinfection in this study is slightly high which is 1.9% and all had chronic liver disease. As the mode of transmission of these viruses are same there is a possibility of coinfection of these viruses among liver disease patients. In patients with HIV infection and hepatitis, the incidence of HCC was higher than in patients with HIV infection without hepatitis (Giordano et al., 2004). In this study most of the cirrhotic patients were found to be positive for HCV hence the chances of developing into a HCC are more likely.

In HCV and HIV coinfected patients SAP and ALT were found to be elevated. Similarly elevation of LFT was found in the HIV positive patients of study conducted by Colin et al., (1999) which also showed that HIV positive patients and HIV negative patients did not behave differently in serum aspartate transaminase activity, bilirubin, prothrombin and histological activity index.
0.7% of this study was found to be coinfected with HCV, HBV and HIV. This could be due to intravenous drug use (IDU). Similar results were obtained in a study where double or triple infection are more often found in patients with the habit of IDU (Sterling and Sulkowski, 2004 & Novick et al., 1988). None of the liver disease patients in this study were found to be coinfected or super infected with all the viruses tested i.e. HCV, HBV, HDV and HIV.

Despite detailed reports documented worldwide in association with HBV, HCV and HIV coinfection, only a few reports have been published regarding these coinfection in India. Kumarasamy (2001) reported a coinfection of HBV (6%) and HCV (4.8%) in HIV infected cases.

HCV ranks significantly behind HBV in the pathogenesis of chronic liver disease in the US, USSR and most Asian countries, except Japan where HCV is the leading cause (Suzuki and Woodfield, 1994). Similarly this study also showed higher incidence of HBV (9.5%) than HCV (5.6%) in liver disease patients. 1.4% of apparently healthy age and sex matched individuals of this study without any signs and symptoms of liver disease were also found to be positive for HBV. This may be due to the fact that the survival rate of HBV in normal environment is greater than HCV or the virulence nature of HBV is greater than HCV. A series of clinical studies from various parts of the world documented natural history of chronic HBV infection particularly in the context of patients showing signs and symptoms of chronic liver disease (Chang et al., 1997).

The present study showed 9.5% of HBV among liver disease patients and 1.5% among controls. Many Indian reports have implicated HBV as the major etiologic agent of chronic liver disease than HCV (Mehta et al., 1992 & Tandon et al., 1996). But most of the HBV positive patients in this study are acute liver disease patients.

With regard to the markers of HBV, 49 liver disease patients were positive for HBsAg followed by Anti-HBc and Anti-HBs. Since HBsAg is the first marker to appear after infection with HBV, its prevalence in this study might found to be high. The overall HBsAg antigenemia among liver disease patients was 24% which is in concordance with findings of other workers in India, with HBsAg detection rate
varying between 12.2%-57% (Dharmadhikari et al., 1990). Four liver disease patients in this study were found to be positive for HBe Ag which indicates the severity of HBV infection. Smith et al., (1976), stated that while the presence of e antigen in carriers of the surface antigen may be of prognostic value. The examination of anti HBe IgM is of importance for differentiating HBV infection in an active phase from its silent phase. Here in this study the prevalence of anti HBe is 0.9% indicating the ongoing viral multiplication among these patients. The detection of anti HBe IgM readily enable the clinician to make the distinction between recent and remote HBV infection (Perrillo et al., 1983). It is thus clear that most of the HBV infection were not remote in this study.

The prevalence of HBV and HDV coinfection in this study is 2.3% indicating the super infection of HDV in HBV patients. Multiple viral infection i.e. coinfection or super infection may be expected more frequently in the Mediterranean area, particularly HBV and HDV reflecting the epidemiological background of the disease (Farci, 2003 & Gaeta et al., 2000). HDV infection was associated with a 3-fold increased risk of HCC as compared with single HBV infected patients (Fattovich et al., 2000).

The prevalence of HIV in liver disease is common (McNair et al., 1992). 5.2% of the liver disease patients of this study had HIV. This prevalence rate is almost similar to the HCV prevalence of this study. This shows the susceptible nature of the patients for blood borne viral infections like HIV and HCV. Overall, 0.4% to more than 50% of HIV patients may carry more than one hepatitis virus (Law et al., 2004, Rockstroh et al., 2004, Lincoln et al., 2003, Sulkowski et al., 2002 and Greub et al., 2000).

The geographic regions, risk groups and type of exposure involved might be the reasons that could be accounted for this high incidence of HIV and HCV. HIV patients with dual hepatitis B and/ or hepatitis C infection have a worse clinical prognosis than HIV mono-infected or coinfected with a single hepatitis virus (Puoti et al., 2000). Previous studies have suggested that the prevalence of HIV and hepatitis is extremely high among patients presenting with emergent conditions (Xeroulis et al., 2005, Sloan et al., 1995 and Kelen et al., 1992).
Generally, in this study all the liver enzymes and pigment tested in liver disease patients were found to be elevated when compared to control samples. This is due to the fact that liver function gets altered or affected in infected cases than the normal individual. In coinfected patients elevation of LFT, particularly ALT was found to be very high than that of single infection. Based on the results of the study conducted by Chu et al., (2002) lower ALT level was found in HBV positive individuals. But in this study ALT levels have increased in almost all the patients infected with one or more than one virus. This is because of the improper function of liver cells due to infection and multiplication of the viruses as they have tropism over liver and virulent. Patients with normal ALT levels have significantly lower inflammation (Shiffman et al., 2006). Most of the chronic liver disease patients with elevated LFT may develop cirrhosis latter. In this study also elevated LFT was found in chronic liver disease cases with and without cirrhosis. However, progression to cirrhosis is slow or absent in patients with persistently normal ALT after 10 years of follow-up (Persico et al., 2006). Fortunately, patients with normal ALT get cured at a rate similar to patients who had elevated ALT (Jacobson et al., 2004).

LFT profile of alcoholic and non alcoholic HCV positive patients of this study showed elevated levels of liver enzymes in both the cases. In alcoholic and non alcoholic HCV and HBV coinfected patients SAP and GGT were found to be highly elevated compared to other enzymes and pigment. This is because alcohol worsen liver function and along with viral infection it can still worsen the situation. Martin et al., (1989) also reported the same.

LFT was also found to be elevated in liver disease patients of this study who were positive for HIV. Bonacini et al., (2004), prospectively followed-up 472 HIV positive patients referred for abnormal liver function tests, 18 (3.8%) of whom had dual or triple hepatitis coinfection and 328 (69.5%) were hepatitis B or C mono-infected. Cribier et al., (1995), observed that mean ALT value of coinfected patients was higher than the HIV negative group but the difference was not statistically significant.

Although HCV infection can be cured in up to 40% of patients, current treatment is not ideal and is associated with a wide spectrum of side effects and complications, leading to a relatively small number of patients being offered therapy.
There is an urgent need for treatment of persons who are coinfected with HCV and other viruses than those with HCV infection alone. Treatment of HCV might improve the tolerability of highly active antiretroviral therapy (HAART) (Sulkowski et al., 2000). Though considerable work has been done on exploring the effectiveness of the plants against HBV, no such report has yet been published on its utility against HCV. The situation, thus demands alternatives and complements to the current therapies (Zitzmann et al., 1999). Due to the side effects associated with the modern day treatments for HCV, three medicinal plants were tested for anti HCV activity in this study.

The angiospermic genus *Boerhavia* (Nyctaginaceae) consists of 40 tropical and subtropical species (Heywood, 1978). Out of these, six species of *Boerhavia* are reported to occur in India (Anonymous, 1948). The root is considered to possess antiviral (Verma and Awasthi, 1979), anticonvulsant (Adesina, 1979), antifibrinolytic (Jain and Khanna, 1989), antibacterial (Olukoya et al., 1993) and hepatoprotective (Rawat et al., 1997) compounds. The plant as a whole is used for oedema and ascites (Anonymous, 1988). This study also proves that *Boerhavia diffusa* has anti HCV IgM binding property and HCV genome inhibition property.

*Eclipta alba* (L.) Hassk. (Asteraceae), a small, branched annual herb, is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases. The alcoholic extract of the plant has shown antiviral activity against Ranikhet disease virus. *Eclipta alba* is widely used in India as a cholagogue and deobstruent in hepatic enlargement. *In-vivo* tests proved that wedelolactone neutralizes the lethal and myotoxic activities of rattlesnake venom (Mors et al., 1989). It is used to treat jaundice and other ailments of the liver and gall bladder (Orning et al., 1980). In this study also extract of *Eclipta alba* showed antiviral activity both by direct and indirect method.

The genus *Phyllanthus* L. of the family Euphorbiaceae consists of about 800 species (Webster, 1994). This species is distributed all over India and is considered as the most widely occurring species of *Phyllanthus* in India (Webster, 1994). *Phyllanthus amarus* also revealed almost similar results like the other two plants tested with regard to anti HCV activity. *Phyllanthus amarus* possibly interrupts the interaction between
HBV enhancer I and cellular transcription factors (Ott et al., 1997). The whole plant extract of *P. niruri* have shown that there exist antiviral properties against HBV (Thyagarajan et al., 1982 & Thyagarajan, 1979). In a clinical trial on chronic HBV carriers, HBsAg clearance in the *P. amarus* treated group was 66%, versus 4% in the placebo group (Thyagarajan et al., 1988). The second open trial in 1990 showed 20% HBsAg clearance and 63.6% loss of infectivity by HBeAg sero-conservation (Thyagarajan et al., 1990).

Investigation on the remedial property of *P. amarus* for HCV is important because, if the plant would possess anti HCV property in addition to anti HBV, which together often infect people chronically (Zitzmann et al., 1999), treatment of both the diseases will be easier. This view may be further strengthened by the fact that the defence mechanism of patients against both the diseases is very similar (Kann and Gerlich, 1998).

Immunomodulators are recommended for people with autoimmune diseases and they are widely used in chronic illness to restore immune system and general health in people who have been on lengthy courses of antibiotics or anti viral therapies. All the three plants tested in this study increased the production of interleukin upon increase in concentration of the extracts. When the immunomodulatory effect of the three plants of this study was seen it was inferred that all the three plant extracts have immunomodulatory effect on human PBMC. Similar results were obtained in a study conducted by Mehrotra et al., (2002).

Adverse hepatic reactions from herbs are well documented and drug-related adverse hepatic events are most frequent (Chan, 2003 & Elvin Lewis, 2001). On the basis of various case reports, liver injury from herbal remedies has ranged from mild elevations of liver enzymes to fulminated liver failure requiring liver transplantation (El Nahhal, 2004). To rule out hepatotoxicity of the plants chosen, all the plant extracts were tested on rat hepatocytes and the results revealed hepatoprotective i.e. none of the extracts were toxic to rat hepatocytes. Jayaram et al., (1994) studying the effect of *P. amarus* on β-galactosamine-induced hepatotoxicity on isolated rat hepatocytes, found that *P. amarus* by itself was not hepatotoxic. The aqueous extract of the dried *Phyllanthus niruri* did not produced any chronic toxicity in mice at 0.2 mg daily dose.
All the three plants chosen for this study are being consumed from the ancient
days. To know whether these three plants are being consumed either for their
nutritional purpose or for their medicinal value the plants were tested for their major &
minor nutrients and secondary metabolites. The results revealed the presence of
appreciable quantity of major and minor nutrients. Qualitative analysis of the plant
extracts for secondary metabolites revealed the presence of flavonoids, terpenoids,
cardinoglycosides and trace amount of steroids. GC-MS analysis of the plant extracts
revealed the presence of various compounds. In *Boerhavia diffusa* thirty compounds
were found. The predominant compound is 3-O-Methyl-d-glucose. Chemical analyses
of aerial parts and roots have revealed the presence of the alkaloids-punarnavine
(Agarwal and Dutt 1936), boeravinone A-F (Lami *et al.*, 1992, 1990 and Kodata *et al*.,
1989), punarnavoside (Jain and Khanna 1989), ursolic acid (Misra and Tiwari 1971)

In *Eclipta alba* forty seven compounds were detected. The predominant
compounds were Z,Z-6,28-Heptatriactontadien-2-one, Phytol and 1,6-Anhydro-á-D-
glucopyranose levoglucosan. Coumestan-type compounds, wedelolactone and dimethyl
wedelolactone, were isolated as the main active principles of *Eclipta alba*, both
constituents exhibiting antihepatotoxic activity (Franca *et al.*, 1995 & Wagner *et
al.*, 1986).

Nineteen compounds were found in *Phyllanthus amarus*. Carissanol dimethyl
ether and bufa-20,22-dinolide 14,15-epoxy-3,16-dihydroxy are the predominant
compounds found. Any one of these compounds alone or in combination may be the
reason for its antiviral activity.

Thus surgeons and public health officials both have a vested interest in the
management of patients infected with the HCV, HBV and HIV. These 3 infections pose
a significant threat to human and to the well-being of healthcare workers who serve
them. The perpetual nature of these infections is augmented by the fact that they are
often asymptomatic. Hence the results of this study can further be explored in
controlling these silent killer viruses particularly HCV by the use of drugs from herbs
without any side effects.