Journal of
Clinical & Cellular Immunology

Roy et al., J Clin Cell Immunol 2012, 3:4
DOI: 10.4172/2155-9899.1000130

Review Article Open Access

Targeting Viral Hepatitis Using Natural Milk Protein and Traditional Medicinal Herbs

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Abstract

Hepatitis is a major health related disease spread worldwide with frequent occurrence of epidemics. It is a zoonotic disease which leads to jaundice, anorexia, malaise and death. Although, vaccines have been developed against hepatitis A and hepatitis B, it is a challenge to generate vaccines against other prevalent forms of hepatitis which are equally harmful and spread worldwide. Natural products that are obtained from living organisms and found freely in nature have proven to be effective against several types of hepatitis due to presence of pharmacologically important bioactive compounds. Since they are natural products they do not cause much harm to body and can be easily applied or consumed. Our main focus is on hepatitis E virus (HEV) which is an opportunistic pathogen and leads to acute jaundice. This virus is mainly present in developing countries with poor sanitation facilities and effects individuals having weak immune response, mainly children, old people, organ transplant patients and pregnant women. HEV infection makes the patient more susceptible to infections from other viruses as well as HIV. In this review, we discussed about the natural protein known as lactoferrin which is isolated from milk colostrum and extracts of some medicinal plants that have proven to be effective against various forms of hepatitis. Such form of natural therapies forms the basis of modern medicine and major pharmaceutical discoveries.

Hepatitis E Virus Epidemiology

Hepatitis E virus (HEV) is a non-enveloped virus that has a single stranded RNA as the genetic material and a genome size of 7.2 kb [1]. HEV causes an enteric non-A non-B acute hepatitis in developing countries due to zoonotic transmission [2,3]. HEV (20%) stands second in the worldwide prevalence [4] when compared to other subtypes of hepatitis, HAV (5%) [5], HBV (33%) (www.hepbe.org/hepb/statistics.htm) and HCV (2%) [6] (Figure 1). Other types of hepatitis are HDV and HFV. There are mainly four major genotypes of HEV out of which 1 and 2 are restricted to human infection, whereas both 3 and 4 are zoonotic [7]. It is difficult to understand the HEV replication cycle with the current HEV cell culture systems however, construction of HEV cDNA clones and replicons [8], development of animal models for structural and functional studies of HEV [9] and the three dimensional structural determination of HEV like particles [10] has helped to understand HEV replication and its molecular biology.

HEV has three open reading frames (ORF) [11]. The ORF1 is 5109 bp long and encodes viral non-structural protein of mass nearly 186 kDa which has four domains; methyltransferase (MeT), papain like cysteine protease (PCP), RNA helicase (Hel) and RNA dependant RNA polymerase (RdRP). Due to improper folding and low expression levels in subgenomic expression systems, the processing of this protein has not been demonstrated [12]. The ORF2 on the other hand encodes a 72 kDa glycoprotein that is a major viral capsid protein [13], the ORF3 is expressed in the early phase of infection and signals cell survival through the activation of extracellular signal regulated kinase (ERK). ORF3 thus encodes for a phosphoprotein involved in cell signalling through MAP kinase pathway [11]. It is proposed that the HEV capsid protein binds to its cellular receptor to initiate the viral entry and replication; however no specific receptors for HEV have been identified so far due to lack of appropriate cell culture systems for HEV. Several binding sites have been identified on HEV such as the C-terminal region of ORF-2 which binds to heat shock cognate protein (HSC70) on cell surface [14] and a dimer of HEV capsid protein (HEV239) which binds to HEV receptors on cells [15]. These features may play an important role in attachment and entry of HEV in the target cells. Studies have revealed that although the HSP90 specific inhibitor (geldanamycin) prevents intracellular passage of HEV239, it does not affect the binding and entry of the capsid protein to the cellular receptors which indicate an important role of HSP90 in HEV infection [16]. Once inside the cell the viral genome is released and the 7-methylguanosine cap structure in 5' NCR of HEV genome regulates the 40S ribosomal subunits to initiate cap-dependent translation of viral proteins and the enzyme involved is RNA dependent DNA polymerase (RdRp) as demonstrated in HEV replication models [17]. It is believed that the ORF-2 proteins

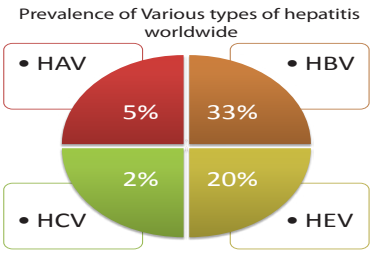

Figure 1: The figure represents the prevalence of various types of hepatitis worldwide. The data shown is after the HAV and HBV vaccines came into use.

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Received September 12, 2012; **Accepted** November 07, 2012; **Published** November 13, 2012

Citation: Roy K, Kanwar RK, Kanwar JR (2012) Targeting Viral Hepatitis Using Natural Milk Protein and Traditional Medicinal Herbs. J Clin Cell Immunol 3:130. doi:10.4172/2155-9899.1000130

plays a major role in assembly and packaging of viral genome [18] whereas the ORF-3 protein is involved in the viral egress [19]. A lot is yet to be known regarding the HEV replication cycle however, from the above mentioned findings the life cycle of HEV can be proposed to a certain extent.

HEV acts as an opportunistic pathogen and its infections are commonly seen in immunocompromised individuals such as children, elderly, patients on immunosuppressive drugs, organ transplant patients and pregnant women [20]. Every year nearly 20 million hepatitis E infections are detected and over 3 million of these patients are found to be suffering from acute case of hepatitis E leading to about 70,000 deaths. Over 60% of all hepatitis E infections and nearly 65% of all HEV related deaths occur in South-East Asia (http://www.who.int/mediacentre/factsheets). Even though the overall mortality rate of HEV is less than 1% worldwide, the mortality rate rises to 20% in case of pregnant women [21,22]. It has been observed that Epstein-barr virus infection increases the susceptibility towards hepatitis infection in immunocompromised and susceptible individuals [23,24].

HEV infections have been reported in veterinarians working with swine and in normal blood donors in United States and other countries [25]. HEV virus infections have been seen in Japan and are presumed to have transmitted through an oral-fecal route mainly due to contaminated drinking water [26]. According to another study, HEV has been found to be widely distributed in New Zealand pig population [27]. HEV variants have been isolated from patients suffering with acute viral hepatitis in Austria [28]. In the year 1978, nearly 52,000 cases of hepatitis E were reported in India which lead to nearly 1700 deaths [29]; followed by HEV epidemic in 1987 in North India where 1273 people were affected [30]. In the year 2005-2006 another HEV epidemic was reported in North India where 3170 cases of jaundice was reported in individuals with mean age 28.8 years. The epidemic lasted for over a year and 18 patients died during the epidemic [31]. Another recent HEV epidemic was reported in Southern India in the year 2008 when nearly 23,915 individuals were affected leading to 315 deaths [32]. Apart from HEV epidemics, India has also suffered with HBV epidemic in 2009 where the mortality rate was recorded to be very high [33]. HEV related epidemics have also been reported in former soviet union, Nepal, Myanmar (Burma), Algeria, Pakistan, Cote d'Ivoire (Ivory coast), Borneo, Sudan and Somalia [34]. HEV infections in pigs have been reported earlier [35] but human HEV infection which was not thought to be widespread has also been reported in USA. The phylogenetic analysis of the HEV US-1 suggested that they may have represented related isolates of a new strain of HEV [36].

Studies have also shown presence of HEV infection in pigs in Australia [37]. All these evidences show that HEV infection is spread worldwide and poses a high risk of transmission of HEV from infected wild and domestic animals to humans. Australia has faced a multistate outbreak of HAV due to semidried tomatoes in the year 2009, when there was a two fold increase in cases of HAV infection when compared to previous years. This outbreak was identified in several Australian states such as Victoria, South Australia, New South Wales, Queensland and Western Australia. The outbreak was extensive and caused a lot of public health damage [38]. Migrants in Australia from various countries have increased over the period of time and this could also be one of the reasons for increased incidences of hepatitis. It has also been found that young adults who have not been vaccinated against HBV are prone to HBV infection in Australia, mainly among people who inject drugs [39]. In the year 2000, over 160,000 patients were diagnosed with HCV infection with 65% patients in the age range 20-39 and an overall male to female ratio of 1.8:1.0 [40,41]. This made HCV infections one of the most common notifiable infectious diseases in Australia.

So far, no antiviral therapy has been shown to efficiently treat HEV infection and there is no specific medicine that can cure hepatitis E infection. It generally suggested avoiding HEV infection by improving the sanitary conditions and patients suffering with HEV are suggested to avoid medicines that can harm the liver, avoid alcohol exercise regularly and drink plenty of fluids (http://hepatitis.emedtv.com/hepatitis-e/hepatitis-e-treatment.html). Thus, where chemotherapy has failed researchers must employ natural therapies which claim to have anti-viral properties. Many dietary supplements and plant extracts that have been consumed by us for ages have been found to have potent antiviral activities that may prove beneficial against the HEV infection.

Antiviral Activity of Natural Milk Protein: Lactoferrin

Colostrum is a rich source of multifunctional proteins which are essential for immunity. One such anti-bacterial, anti-parasitic, anti-cancer, anti-oxidant and anti-viral protein is lactoferrin (Lf) which acts as the first line of body's defense [42]. It consists of a single polypeptide chain of 80 kDa with two globular lobes containing an iron binding site. The multifunctional nature of lactoferrin is explained by its ability to protect from pathogens, induction of apoptosis in cancer, inhibiting cancer proliferation and restoring immune cells in body after chemotherapy [43]. Lactoferrin is folded into N and C lobes each of which comprises of iron binding sites and normally lactoferrin is only 15% saturated with iron, this form of lactoferrin is termed as native lactoferrin (Native bLf). The lesser iron saturated form of lactoferrin where the protein is only 4% saturated with iron is termed as apo-lactoferrin (Apo-bLf) and the form of protein where iron saturation is more than 50% is termed as iron saturated lactoferrin (Fe-bLf) [43]. Both Apo-bLf and Fe-bLf have shown to have anti-oxidative effects and have shown to induce apoptosis in cancer cells [44]. Fe-bLf has shown to significantly reduce tumour vascularity, increased anti-tumour cytotoxicity, tumour apoptosis and infiltration of tumours by leukocytes [43]. We prepared another form of lactoferrin termed as selenium saturated bovine lactoferrin (Se-bLf), where 98% selenium saturation was achieved. Due to the availability of both selenium and bLF in whole milk apart from being non-cytotoxic to normal body cells, Se-bLF is a promising natural dietary supplement. It also enhances the body immune system and has a potential for being used as a chemo-preventive agent against various forms of cancers [45].

Lactoferrin and its peptides have been used for the treatment of hypertension, thrombosis, immunodeficient disorders, dental diseases and asthma [46]. The versatility of this molecule has made it one of the most important biological molecules that is being studied and researched on for anti-cancer therapies. Orally administered lactoferrin has shown to stimulate cytokine production, increase natural killer cell, macrophage, dendritic cells and cytotoxic T cell activity [43,47]. Lactoferrin binds directly to most immune cells and has immuno-modulatory and regulatory activity. Lactoferrin has shown to inhibit colorectal, lung, skin, bladder, oesophageal and breast cancer cells [43,48]. Lactoferrin has been found to be released into circulation by the activated neutrophils during an inflammatory response. This lactoferrin is responsible for iron regulation and inhibition of interleukins and tumour necrosis factor [49]. Lactoferrin has also shown significant activity against enteric pathogens such as *Salmonella*, *Shigella* and *E.coli* [50]. It inhibits their growth and impairs the virulence factors, decreasing their ability to adhere and invade the mammalian cells. Lactoferrin has shown bactericidal effects against pathogenic bacteria such as *Vibrio cholera* and *Streptococcus mutans*,

the reason for this activity is the ability of lactoferrin to scavenge iron which is essential for bacterial growth [51].

Although, several constituents in milk have potential antiviral effects such as lysozyme, lactoperoxidase, IgM, IgG but studies have shown lactoferrin on its own has direct antiviral activity [52]. Antiviral activity of human lactoferrin and transferrin has been established against both DNA and RNA viruses [52] such as the friend virus complex (FVC), a murine retrovirus [53] hepatitis C virus (HCV) [54], rotavirus [55], poliovirus [56], respiratory syncytial virus (RSV) [57], human immunodeficiency virus (HIV) [58,59], herpes simplex virus (HSV) [60] and cytomegalovirus [59] (Table 1). There are mainly two envelope proteins present in hepatitis C virus (E1 and E2) which exist as hetero-oligomer. A study has reported that bLf specifically binds to both E1 and E2 *in vitro* and forms complexes with HCV both in cell culture (HepG2 cells) and in cell free supernatants [54] it is also known to bind directly to the rotavirus and inhibit the rotavirus infection on target cells [61]. Thus, lactoferrin interferes with adsorption of HCV on cell surface. Hepatitis E virus is a non-enveloped virus but it is proposed that it enters the cells by binding to the lipoproteins present on the cell surface, lactoferrin is also known to bind to these lipoproteins and repel the virus [52]. Studies have also shown that lactoferrin has suppressed the viral growth by inhibiting the viral replication after entry of the HIV (type 1) virus into the target cells [62] (Figure 2). Another important aspect of the antiviral activity of lactoferrin is that it acts as an immune boost by activating the immune cells of the body such as natural killer cells, granulocytes and macrophages which play a major role in combating the infection during early stages of viral infection [63]. No studies have been conducted to observe the effects of lactoferrin and its saturated forms on HEV, however work done on other hepatitis viruses and other similar viruses show the importance of milk proteins and lactoferrin as an antiviral therapeutic.

Significance of Camel Lactoferrin

Lactoferrin and its various forms have been proven to be effective against various types of hepatitis. Zinc (Zn) saturated bovine lactoferrin has shown higher degree of inhibition of HBV DNA *in vitro* when compared to iron (Fe) saturated and manganese (Mn) saturated lactoferrin [64,65]. A study has shown that bovine lactoferrin in hepatitis C virus patients produced a Th1 cytokine response for up to 3 months that favours the eradication of HCV by interferon (IFN) therapy in cultured hepatocytes (CHC) [66,67]. According to a report from 1985, there are 1.1 million camels in India which accounts for nearly 6.3% of the world camel population [68]. Reports also claim for presence of over 1 million camels in Australia, which are spread across Western Australia, South Australia, Northern Territory and Western Queensland [69]. It has been found that concentration of lactoferrin found in mastitic camels is much higher than normal camels [70] and concentration of lactoferrin in cow's milk is lower than human's milk [71]. When compared to lactoferrin concentration in bovine milk (140 mg/l), the lactoferrin content in camel milk is much higher (220 mg/l) [72]. Lactoferrin from camel has been found to display the characteristic functions of iron binding and release of lactoferrin as well as transferrin simultaneously. Thus, camel lactoferrin possess features of both lactoferrin and transferrin which has not been seen in lactoferrin isolated from any other source [73]. The formation of disulphide patterns in camel lactoferrin is identical with human lactoferrin but few sites such as the glycosylation site are entirely different, thus camel lactoferrin resembles the human lactoferrin to a sequence identity of 70% [73]. HCV which is a major worldwide health risk has no effective treatment till now. It has been found that camel lactoferrin which is chemically similar to human lactoferrin was able to prevent HCV infection and proved to have effective antiviral activity against HCV [74,75]. Although human lactoferrin is the most heat-resistant, it is the camel lactoferrin which has maximum antibacterial potential [76]. Due to the high camel populations present in both India and Australia and the potential benefits of camel lactoferrin over other forms of lactoferrin, camels farms could supply high quantity of lactoferrin in both countries which could be used for treating various forms of hepatitis in both the countries and worldwide.

Antiviral Medicinal Plants

The basis of all sophisticated traditional medicines is plants as they have provided various bioactive compounds and plant products since thousands of years [77-79]. Medicinal plants have been used for their antiviral properties since the beginning of human civilization depending on the type of viral infections [80]. The medicinal plants have several bioactive compounds which exhibit antiviral properties such as flavonoids [81], terpenoids, lignoids [82], alkaloids and phenolic acids [83]. Flavonoids are natural phenolic compounds mainly found in fruits, vegetables, grains, bark of tress, roots, stems,

Sl No	Virus Type	Antiviral Mechanism	Reference
1	Friend virus complex (FVC)	No direct effect on FVC infection but Lf decreases myelopoiesis in bone marrow and spleen	[53]
2	Hepatitis C virus (HCV)	Binds to envelope protein of HCV (E1 and E2) and prevents adsorption of HCV to target cells	[54]
3	Rotavirus	Prevents docking of virus to viral receptors on the target cells	[55]
4	Poliovirus	LF interferes with entry of poliovirus into the target cell	[56]
5	Respiratory syncytial virus (RSV)	Not been elucidated so far	[57]
6	Human immunodeficiency virus (HIV)	Lf binds to gp120 and prevents adsorption and entry of HIV	[58,59]
7	Herpes simplex virus (HSV)	LF inhibits adsorption of virus to the target cells	[60]
8	Cytomegalovirus	LF interferes with the entry of virus into the target cell	[59]

Table 1: Antiviral activity of Lactoferrin.

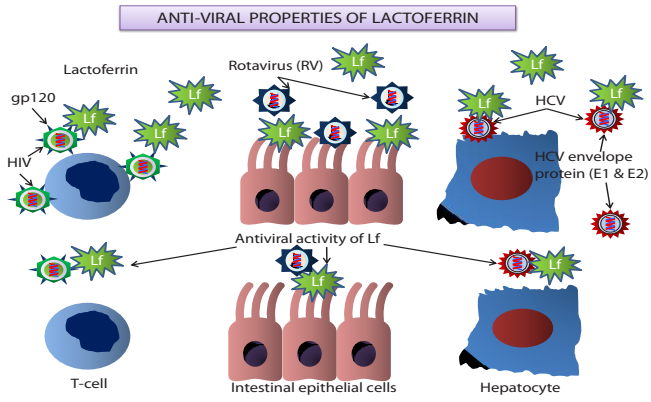

Figure 2: The anti-viral properties of natural milk protein: lactoferrin is demonstrated in the figure 2 where lactoferrin binds to gp120 protein of HIV and prevents attachment of HIV to target cells; lactoferrin also binds to the target site of rotavirus (RV) present on the intestinal epithelial cells and blocks the attachment of the RV to the target. Lactoferrin is found to bind to the envelope proteins (E1 and E2) present on the HCV surface and modify them in such a way that the HCV fails to attach to its receptors on the target cells.

flowers, tea and wine [84]. Most of the antibacterial, antitumour, antiparasitic and antiviral activity associated with flavonoids is due to their antioxidant activity [85], Studies have shown the antiviral activity of flavonoids on herpes simplex virus, respiratory syncytial virus, parainfluenza virus and adenovirus [86], Flavonoids have also been established as an anti-HIV agents as they have shown to inhibit the activity of reverse transcriptase or RNA directed DNA polymerase [87]. Terpenoids have been evaluated for their antiviral activity against SARS and have shown significant inhibition of the virus [82], antiviral activity of several alkaloids and phenolic acids have been tested against DNA virus herpes simplex type 1 and RNA virus parainfluenza type 3. The compounds atropine and gallic acid showed potent antiviral effects against both these viruses [83].

Antiviral effects of medicinal plants have been reported against various viruses such as the influenza virus [88], herpes simplex virus type 2 (HSV-2) [89], human immunodeficiency virus (HIV) [90,91], hepatitis B virus [92] and severe acute respiratory syndrome (SARS) virus [93]. The extract obtained from the leaves of *Warscewiczia coccinea* has shown significant antiviral activity on HBV and HIV-1 replication [94]. Herbal extracts of various medicinal plants have proven to be successful against the hepatitis virus. Extracts of *Acacia nilotica* [95], *Solanum nigrum* [96] and *Phyllanthus amarus* [97] have shown significant inhibition of the HCV whereas, extracts of *Phyllanthus urinaria* has proven to successfully inhibit HBV [98] (Figure 3). Another study performed with extract of *Embelia schimperi* showed presence of two important bioactive compounds; embelin and 5-o-methylembelin that showed significant inhibition of HCV protease [99] (Table 2). Some other medicinal plants that have shown promising antiviral activity are *Dryopterisfilix-mas* which have tannic acid and inhibit viral replication of influenza A virus. *Chamaecyparis lawsoniana* which has several phenolic compounds and inhibits herpes simplex virus (HSV). *Combretum molle* which has punicalagin and inhibits replication of HIV-1 and HIV-2 viruses. *Momordica charantia* which has ribosome inactivating proteins (RIPs) and prevents infection and replication of both HSV and HIV. *Boehmeria nivea* which inhibits HBV DNA and *Trifolium* species that have secomet V which inhibits replication of HIV, poxvirus and SARS virus.

Recent findings from our group have shown antiviral effects of commonly used medicinal herbs against the hepatitis E virus. This was the first study done to see the antiviral effects of medicinal plants against HEV in cell culture model. Some of these extracts are commonly used for their antibacterial properties but have never been tested for their antiviral properties. The plant extracts were obtained from *Kaempfaria galanga* (Galangal), *Mimosa pudica* (Touch me not), *Coleus aromaticus* (Doddapatre) and *Paederia foetida* (Stinkvine) and tested for cytotoxic

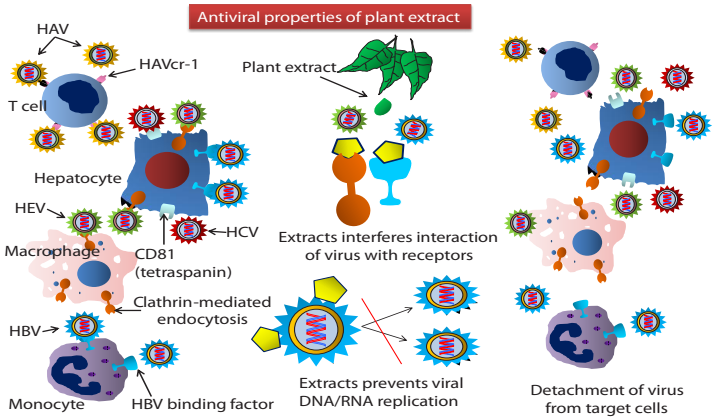

Figure 3: The figure 3 shows the various receptors via which the subtypes of hepatitis virus enter the target cells. The presence of medicinal plant extracts prevents the entry of the viruses by binding to their specific receptors or directly inhibits the viral RNA and its replication.

Sl No	Plant Species	Bioactive compounds	Virus Type	Molecular function	Reference
1	*Warscewiczia coccinea*	Unknown	Hepatitis B Virus (HBV) and Human Immuno deficiency Virus-1 (HIV-1)	Inhibits Viral replication	[94]
2	*Acacia nilotica*	Unknown	HCV	Inhibits viral RNA	[95]
3	*Solanum nigrum*	Flavonoids, Saponins, and Phytosterols	HCV	Inhibition of NS3 region of HCV	[96]
4	*Phyllanthus amarus*	Ellagic acid	HCV	Inhibits Viral polymerases	[97]
5	*Phyllanthus urinara*	Ellagic acid	HBV	Inhibits Viral polymerases	[98]
6	*Embelia schimperi*	Saponin	Hepatitis C Virus	Inhibition of HCV protease and suppression of cytokine signalling 2 pathway	[99]
7	*Dryopteris filix-mas*	Tannic acid	Influenza A virus	Viral replication inhibition	[88]
8	*Chamaecyparis lawsoniana*	Phenolic compounds	Herpes simplex virus (HSV)	Unknown	[89]
9	*Combretum molle*	Punicalgin	HIV-1 and HIV-2	Inhibition of HIV replication	[90]
10	*Momordica charantia*	Ribosome inactivating proteins (RIPs)	HIV and HSV	Infection and replication of both HSV and HIV	[91]
11	*Boehmeria nivea*	Unknown	HBV	Inhibition of HBV DNA	[92]
12	*Trifolium species*	Secomet V	Anti-HIV, anti-Poxvirus, and anti-SARS activity	Inhibition of viral replication	[93]

Table 2: Antiviral medicinal herbs.

effects on porcine epithelial (CLAB) and porcine macrophage cell lines (PoM_2). We found that the extracts of *Mimosa pudica* and *Paederia foetida* had proliferating effects on porcine epithelial cells whereas the extracts of *Kaempfaria galanga* showed proliferation of porcine macrophages. When tested for their antiviral activity against HEV infected CLAB and PoM_2 cells, the survival and growth of the HEV infected cells was significantly enhanced after treating with extracts from *Mimosa pudica* (1.39 fold)and *Kaempfaria galanga* (1.57 fold) when compared to the untreated cells (Figure 4). Thus, from our results it can be inferred that the extracts from the medicinal plants *Mimosa pudica* and *Kaempfaria galanga* are able to prevent the binding of these viruses with their specific receptors and thus aid in prolonging the cell viability. It is still unclear whether the extracts are inhibiting the virus itself. Another possibility could be that the extracts were able to downregulate the expression of different receptors on the surface of cells which are responsible for attachment and receptor mediated internalization of virus particles. Further studies are required to understand the antiviral effects of these medicinal plant extracts on binding of the virus to its specific receptors and on the virus itself [100].

Nanomedicine Against Hepatitis

Nanomedicine is the employment of nanoparticles for medicinal delivery where the desired drugs are bound or encapsulated with nanoparticles synthesised from various polymers or materials and delivered into the target cells [101]. Nanomedicine has recently come up as the most optimum method for drug delivery as it has gained a lot of patient compliance. Nanotherapy reduces side effects, cytotoxicity of the drugs and helps in sustained drug release which removes the option of multiple drug dosage. The need for nanotherapy in delivery of antiviral therapeutics is to localize and target them in order to obtain maximum inhibitory effects on the viral particles. The nanoparticles can be targeted using various targeting ligands such as antibodies, aptamers and peptides which are specific towards target proteins [102,103].

Most of the nanoparticle related studies done on hepatitis have been performed on HBV or HCV [104]. Several types of nanoparticles have been used to deliver DNA or siRNA in order to inhibit the hepatitis infection. Chitosan nanoparticles have been used to deliver plasmid DNA encoding surface protein of HBV in HeLa cells in order to induce humoral and cellular immune response. The immunization studies were conducted in BALB/c mice for measurement of specific IgG and IgA response. Nasal administration of the nanoparticles resulted in high anti-HBsAg titre whereas intramuscular administration did not elicit significant IgA titre. The advantage of using chitosan nanoparticles was that it protected the plasmid DNA from degradation as they were stable at physiological pH. It was proposed that the small plasmid-chitosan nanoparticles were able to pass through the membrane and reach the underlying lymphoid tissue where they interacted with the antigen presenting cells and enhanced the immune response [105]. Another study involved the collection of serum samples from chronic HCV patients and the detection of HCV RNA using unmodified gold nanoparticles. These nanoparticles when added to HCV RNA changes the colour of solution from red to blue within 1 minute with 92% sensitivity. Thus, a system was developed to detect HCV RNA using unmodified gold nanoparticles in biological samples with high specificity [106]. Dextran coated magnetic iron oxide nanoparticles have been used to deliver DNAzymes in human hepatoma cell line (Huh-7) for treatment of hepatitis C by inducing knockdown of HCV gene NS3. The proteins encoded by these genes are essential for the virus replication. The *in vivo* studies showed that the nanoparticles were taken up by both hepatocytes and kupffer cells which are essential for treatment of HCV [107]. In another study siRNA (specific for inhibition of HBV gene expression and viral replication) was condensed with cationic liposomes to form anti-HBV formulation which was further coated by polyethylene glycol (PEG). This strategy resulted in HBV replication knockdown in both cell culture and murine models. The systemic administration of the PEG coated siRNA loaded liposomes led to suppression of HBV replication in HBV transgenic mice up-to 3 fold. The advantages of using liposomes was that they could be loaded with more quantity of siRNA and siRNA doses as low as 0.01 mg/kg body weight of mice were able to silence the target gene [108]. M-Cell targeted biodegradable PLGA nanoparticles were synthesized in another study for oral immunization against hepatitis B. The lectinized nanoparticles successfully induced enhanced immune response. These nanoparticles were loaded with HBsAg and Ulexeuropaeus (UEA-1) lectin was anchored to the nanoparticles to target them to the M-cells of peyer's patches. The nanoparticles successfully induced high serum anti-HBsAg titres and induced mucosal immunity by enhancing the IgA levels in salivary, intestinal and vaginal secretions. The secretion of cytokines such as IL-2, IFN-γ levels in spleen was also found to be elevated [109]. Thus, use of various nanocarriers depending on the target and the drug have proven to be highly successful both in inhibiting the hepatitis virus replication and in inducing high levels of immune response which acts as body's defense against the viral infection.

Both lactoferrin and plant extracts are derived from natural sources and are proved to be non-cytotoxic to humans on consumption, it is not sure that they can specifically target the hepatitis virus on their own. Since, hepatocytes are the key site of hepatitis viral infection, a novel system is required to target the viruses and deliver the lactoferrin and the medicinal plant extracts to the target site. In our lab we have designed and developed chitosan coated calcium phosphate nanoparticles encapsulated in alginate gel (AEC-Ch-CP). All the constituents of these nanoparticles are established to be biocompatible. Chitosan and alginate are known biodegradable polymers with negligible immunogenicity and biocompatibility [110] whereas, calcium phosphate is an important mineral constituent found in the human body. Fe-bLf has been successfully delivered orally using these nanoparticles and the pH sustainability studies revealed successful protection induced by alginate coating to the inner layers of chitosan and calcium phosphate loaded by Fe-bLf. The bio-distribution studies have shown significantly higher release of Fe-bLf and nanoparticle concentration in liver when compared to spleen, kidney, lung, heart, brain and stomach [111].

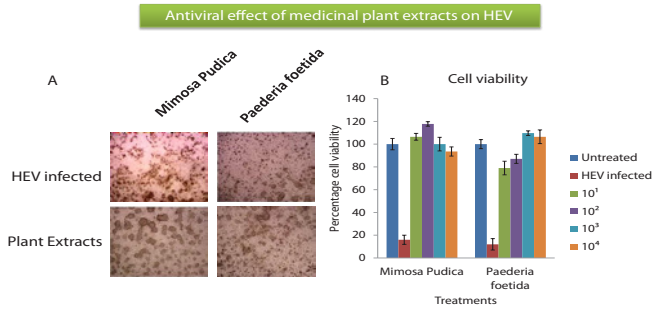

Figure 4: A) Representative images of HEV infected POM_2 (Porcine macrophage cells) showing cell death due to HEV infection. Once treated with the medicinal plant extracts the HEV infected POM_2 cells showed enhanced viability, B) The graphical representation of cell viability in HEV infected POM_2 cells after treatment with various dilutions of the medicinal plant extracts.

Expert Opinion

Plant sources and natural products have been used for medicinal purposes, consumption and various other reasons since ancient times. Even though several medicinal herbs have proved to be significantly effective against various pathogenic viruses, the modern medicine relies on synthetic drugs. However, this review has tried to focus on the antiviral medicinal herbs and the antiviral properties of a natural milk protein: lactoferrin. These natural remedies have proved to be successful against various bacterial, viral and parasitic infections *in vitro* and *in vivo* and thus deserve an opportunity to be tried on a larger scale for treatment of major pathogenic viruses which are difficult to contain in a specific landmass such as the hepatitis virus. The milk protein lactoferrin and the extracts from the medicinal plants are being used as dietary supplements since ages and hence are well known to be non-toxic to normal human system. Hepatitis which has caused epidemics throughout the world time and again must be dealt with using such natural therapies as not only they are non-cytotoxic but they also boost the immune response of the body. Nanomedicine has made it possible to selectively target several infectious viruses in recent times. Various nanoparticle systems have been developed and are found to be successful against these pathogenic viruses. Thus, a combined nanoformulation of both lactoferrin and the medicinal plant extracts could work in a synergistic manner to eradicate hepatitis from the world.

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