The worldwide problem of Hepatitis B is the common serious liver necroinflammatory infection, and it is referred as silent killer initiated by Hepatitis B Virus (HBV) which slowly damages the liver. According to the World Health Organization (WHO), Hepatitis B is a potentially life-threatening liver infection caused by Hepatitis B virus.

Viral infections causes acute and chronic inflammatory diseases of liver and, in some cases, develops into serious complication such as hepatocellular carcinoma or liver cirrhosis or chronic liver disorders. Now a days, millions of people suffer from chronic HBV globally [Aguilar *et al.,* 2004; Chisari *et al.,* 2010]. Hepatitis B generally affects the healthy workers, so that, it requires a nontoxic and effective vaccine.

The vaccine for Hepatitis B has been accessible since 1982 but currently available vaccines are effective in preventing the viral infection by 95% and the chances of development of liver cancer still exist. Liver cancer is due to the presence of Hepatitis B infection mostly in chronic stage. According to the data, 90% children are chronically infected with Hepatitis B virus (HBV) by birth and 5-10 % are newly Hepatitis B infected adults having chronic disease. Those people infected by birth or in early childhood, are having higher chances of chronic Hepatitis B virus because the immune system is not fully developed. Chronic Hepatitis B virus infection is directly proportional to the age and time of first exposure. When cellular immunity is pharmacologically restrained, Hepatitis B virus grows rapidly and replicates continuously at high levels, resulting in chronic cytological abnormalities or inflammation in liver tissues [Guidotti *et al.,* 1995; Jaganathan and Vyas, 2006]. Lack of T-cell adaptive immune response and specific humoral immune response is another reason of chronic HBV infection [WHO 2014]. As per literature, for the generation and

production of protective immune system, both the adaptive as well as innate immune systems are required [Janeway *et al.,* 2005].

Presently, no well-established treatment is available for the chronic Hepatitis B virus infection; the available treatment is based on induction of host mediated immunological control and reduction of HBV-DNA levels in blood serum. The main focus on treatment of Hepatitis B is reduction of any histological inflammation and control of disease progress. Current Hepatitis B virus treatment includes antiviral interferon therapy, nucleos(t)ides or nucleoside analogs therapy and immunotherapy, which suppresses viral replication but not the induction of any protective immune response or viral clearance in chronic infection [Lindh *et al.,* 2008; Scaglione and Lok, 2012].

Interferon therapy, like alpha interferon, is only effective in less than 50% of virus carriers [Hoofnagle *et al.,* 1987]. A long-term treatment may be troubled by escape mutants, development of drug resistance, several side effects and heavy economic burdens [Chen, 2010].

Nucleos(t)ide or nucleoside analogues like adefovir, tenofovir, telbivudine, entecavir and lamivudine, mainly increases the CD8+ T cell response [Chien and Liaw, 2008] and directly suppress the Hepatitis B virus replication by inhibiting the virus polymerase/reverse transcriptase. However, these are not effective in eliminating the virus [Bocher *et al.,* 2000; Di Bisceglie *et al.,* 2002]. The major concern of this therapy is also the development of drug resistance and side effects.

The most recent long term potential approach to eliminate the virus is immunotherapy. It works to stimulate an adaptive immune response through immunization or vaccination or inoculation. Vaccine is made by modified or killed microorganisms like

bacteria or viruses, which develops resistance for specific disease. It does not promote the cause of disease but builds a protective immune system, which continuously protect against the disease [Hilleman, 2001; Nandedkar, 2009]. The immunization or vaccination is not the treatment and cure for the chronic infection and not even the treatment of infection.

The more appropriate approache of curing Hepatitis B virus infection is manipulation of host immune system and clearance of virus but manipulation of host immune system is very difficult. Some time it creates major problems such as cirrhosis, liver cancer and finally death.

To overcome these problems and limitations of currently available therapies, a new nanovaccine or nanoparticulate system against the Hepatitis B Virus infection was developed. The first developed nanovaccine was self-assembling nanoparticles, having modified adenoviruses, which deliver the target gene. With the help of this gene, cells produce some special protein which activates the immune response system. A nanovaccine is synthesised using polymer or polysaccharide which targets special protein molecules and adhere to the protein module like carbohydrate binding domain, in which antigens are either bound to the surface of polymer or encapsulated inside the vehicle by covalent or non-covalent attachment [Nobs *et al.*, 2004; Zolnik *et al.*, 2010].

In the past decades, the application of nanotechnology in virology has been increased tremendously. Nanoparticles having different compositions, various sizes, and surface morphology properties were developed [Mamo and Poland, 2012]. Nanoparticles based approach is revolutionizing the diagnosis of disease as well as the delivery of active substance for the treatment and prevention of disease [Couvreur and Vauthier, 2006]. It produces prophylactic as well as therapeutic way to enhance antigen antibody immunity

and deliver the therapeutic molecules at the target site [Correia-Pinto *et al.,* 2006; Roldao *et al.,* 2010].

The mechanism of antigen delivery through nanoparticles involved direct entry into living cells followed by cellular endocytosis, particularly pinocytosis due to their size similarity to cellular constituents [Tissot *et al.,* 2008; Treuel *et al.,* 2013]. It has unique property (like adjuvant) of enhancing the immune response. The main *in-vivo* performance of nanoparticles depends upon their hydrophobicity, surface modification, surface charge, and particle size [Kumari *et al.,* 2010].

Hydrophobicity of the nanoparticles affects the rate of release of antigen from the nanoparticles and increases *in-vivo* circulation time inside the body. Antigen loaded biodegradable polymer delivery vehicles are hydrophobic in nature that inhibit water from penetrating into the polymer matrix, therefore slowly degraded, rapidly opsonized and delayed the release [Seong and Matzinger, 2004]. Hydrophilic parts of polymer (glycol moiety) enhance the circulation time. Nanoparticles with sizes less than 1000 nm have the important properties for vaccine delivery they can easily penetrate into capillaries and tissue gaps to reach the target organs. Thus, the subcellular size and ready internalization provides controlled release of antigen and ensure greater stability and improved utility [Liu *et al.*, 2008].

Vaccine is administered intramuscularly in deltoid muscle for the treatment of Hepatitis B infection. The standard adult regimen of Hepatitis B vaccine is 20 µg (40 µg for dialysis patients) administered at intervals of 0, 1 and 6 months (booster dose). Due to administration and deposition in the subcutaneous tissue, direct vaccination showed lower immune response [Wahl and Hermodsson, 1987; Shaw *et al.,* 1989; Zuckerman *et al.*, 1992]. Therefore, booster dose is required. When a person skips his booster dose, it

increases the chances of acute as well as chronic virus infection. In some cases, it leads to development of immune tolerance, thus the immune system is not adequate to protect against the viral attack. In these cases, the host immune system is not sufficient to protect against this virus or foreign materials. Persistence of HBV infection leads to increased amount of virus particles or HBsAg in blood and liver. Consequently, in chronic condition, immune tolerance develops in patients [Patterson *et al.,* 2001]. Therefore, for the successful immunization and superior targeting to cellular and molecular levels, nanocarrier-based vaccines need to be developed. Nanovaccine formulation delivery system maintains sustained release of antigen and also protects the antigen from *in-vivo* environmental conditions by encapsulation. Changes in physicochemical characteristics of the nanoparticles influence the interaction of nanoparticles with immune cells and ultimately affect the immunomodulating actions [Yao *et al.,* 2007; Sekhon and Saluja, 2011].

Pawar *et al* (2010), showed development of HBsAg loaded microspheres/microparticles with enhanced activity. However, microspheres/microparticles require additional adjuvant (alum) to stimulate immunogenicity [Yao *et al.,* 2007; Zolnik *et al.,* 2010]. Nanocarriers have several advantages over microsphere such as long term immunogenicity, biocompatibility and biodegradability [Akagi and Akashi, 2006]. Further, slow degradation of polymer also results in sustained release of antigen from polymeric nanoparticles [Danhier et al., 2012]. Considering all these advantages, it was proposed to develop a nanotechnology based nanovaccine/ nanoparticles antigen delivery system to promote the antibody production and eliminate booster dosing. Thus, it was planned to prepare HBsAg loaded nanoparticles and conduct both *in-vitro* and *in-vivo* evaluation of the prepared nanoparticles in BALB/c and humanized xenograft mice

to examine the efficiency of developed nanovaccine for the treatment of the acute as well as chronic Hepatitis B virus infection.
