Chapter-I

INTRODUCTION AND REVIEW

Immunostimulants, which are also known as immunostimulators are substances comprising of drugs and nutrients that stimulate the immune system by inducing activation, or increasing activity of any of its components. During the last two decades very intensive investigations are carried out for the production of a novel category of biologically active substances known to the world as “Immunostimulants”. They are the products derived from natural or synthetic origin with different chemical characteristics and varied modes and mechanism of action (Petrunov et al., 2007). Immunostimulants activate different elements and mechanisms of the immune system of humans and animals, to re-inforce the body’s natural resistance in order to successfully and amiably cope with various viral and bacterial infections or to help in the treatment of disastrous and chronic ailments and severe immune-suppression. The immunostimulants promote phagocytosis, activates properdin and complement systems, and helps in the release of interferons like $\alpha$ and $\gamma$. Immunostimulants promote synthesis of specific antibodies and cytokines and represent an emerging class of drugs for the treatment of infectious disorders. The two main categories of immunostimulants include:

(i) Specific immunostimulants are those which provide antigenic specificity in immune response such as vaccines or any antigen.

(ii) Non specific immunostimulants are those which act irrespective of antigenic specificity to augment immune response of other antigen or stimulate components of the immune system without antigenic specificity, such as adjuvants and non-specific immunostimulators.
Many chemical substances produced by the human body function as immunostimulants. Cytokines are a type of immunostimulants which are produced by the cells of the immune system and may have an enhancing effect on immune system. Female sex hormones and growth hormones are known to have immunostimulant effect. Immunostimulants may be the nutrients substances obtained from food or medicinal herbs that bounces the immune response. This class of immunostimulants includes several vitamins, minerals, antioxidants and probiotics etc. Beta-glucans are another type of nutrient immunostimulants found in plants, fungi and bacteria. Various types of beta-glucans occur in cellulose fibers in trees and plants, yeast and certain strains of bacteria and fungi. Beta-glucans have been used as adjuvants in cancer therapy. Immunostimulants activate both non-specific and specific defense mechanisms, cell mediated immunity and specific immune responses. Immunostimulants can be administered before, with or after vaccines to amplify the specific immune response in generating elevations of humoral anti body levels and the number of Anti-Body Secreting Cells (ASC) (Siwiki et al., 2010). Special applications of immunostimulants include assisting spray or other regimens to increase the topical uptake of vaccines. Combined use of vaccines and immunostimulants is emerging as one of the innovative approaches in adjuvant development in fish (Gautam et al., 2008).

Basing on these indispensable criteria, that because of the lack of a specific treatment and a specific vaccine for many infectious diseases in a broad spectrum visage, it is quite reasonable to consider the non-specific immunotherapy and the immunoprophylaxis based on the use of some Immunostimulants reinforcing the natural immune mechanisms as a very promising approach. It has long been established that
exogenous Immunostimulants can enhance the host defense system. The use of such immunoadjuvants in most cases has often been proven non-specific, hence limiting their capabilities in fighting against specific foreign invasion, particularly against tumor cells which in most cases can easily evade the host immune surveillance system (Wei et al., 2009). Immunostimulants represent an emerging class of drugs for the treatment of infectious disorders and cancer. CpG oligonucleotides and imiquimod, prototypic drugs in this category, are known to activate dendrite cells (DCs). The recent medical findings throw a magnanimous light for choosing the Immunostimulant drugs over the administration of immunosuppressant drugs in order to cure auto immune diseases. The usage of immunostimulants is much promising than the utilisation of steroids and other alternatives. Immunomodulation therapies with the usage of immunostimulants promises to be an effective prophylactic and therapeutic modality for chronic and recurrent respiratory infections (Blanca et al., 2007). One of the main obstacles in the development of immunostimulants is the poor understanding of the mechanism of action. Except for some compounds the mechanism of action of bacterial products is not well understood. Some appear to act through activation of monocytes and macrophages and enhancement of polyclonal proliferation of B-cells.

Currently immunostimulation has become a more widely discussed method of managing patients suffering from infectious disease. An ideal situation when a host is exposed to pathogen challenge (e.g. bacteria or virus) is to have optimal immunity that protects the host from disease. Immunity towards bacteria might be achieved, as a result of natural process following infection, or as a consequence of medical intervention including vaccination, administration of immunoglobullins or therapy with
immunostimulants derived from bacteria (Adriana Rozy et al., 2008). Bacterial immunostimulants containing lysate or components of bacterial cells (ribosomal extracts) were shown to induce a non-specific response (i.e. intensification of phagocytes) but also to orchestrate both cellular stimulation of B-cells and T-cells and humoral responses (antibodies and proinflammatory cytokines production). The compensatory effect of a bacterial lysate known commercially as Broncho-Vaxom (BV) is a bacterial immunostimulant; when challenged on the immunosuppressive action of cyclophosphamide, the cyclophosphamide induced immunesuppressed mice when treated with Broncho-Vaxom, recovered the normal IgM and IgG levels in serum. Furthermore, normal cell proliferation and increase in its size was clearly observed in thymus indicating heightened level of immune response (Bosch et al., 1984). As an ideal bacterial immunostimulant, it is proved to influence both the innate (influencing the activation of macrophages and neutrophils) and acquired immunity (production of cytokinins). HemoHIM is a new herbal immunostimulant, when administered to the aged mice, it resulted in the complete restoration and proliferative response of cytokines in spleen, this novel product has been proved to be an ideal supplement for immune restoration in aged persons (Park et al., 2008). Another immunostimulative therapeutic agent of bacterial origin deduced from E. coli strains by hame OM-89 (uru-vaxom) is shown to provide protection against prevailing/recurrent urinary tract infections in humans; and certain bacterial infections in mice.

In many cases, specific therapy in the form of antibacterial, anti-fungal, antiprotozoal or anti-parasitic therapy will work in combination with the immune system to aid with pathogen clearance. In some instances, the addition of an immunostimulant
will aid in “boosting” the immune response so that a robust immune response may act in coordination with the antimicrobial drug to clear the infection. Usage of immunostimulant drugs is a modern technique in medical science known as immunomodulation. An immunomodulator may be defined as a substance biological or synthetic, which can stimulate, suppress or modulate any of the components of the immune system. Immunomodulators are immunoadjuvants, immunostimulants and immunosuppressants. Immunoadjuvants are used for enhancing efficacy of vaccines and therefore, could be considered as immune stimulants. In recent years, liposomes and other agents are being manufactured as an alternative. Immunostimulants are envisaged to enhance the body’s defense mechanism against various infectious factors - allergy, autoimmunity and cancer. In healthy individuals, the immunostimulants are expected to serve as prophylactic or promotive agents acting as immune-potentiators by enhancing the basal levels of immune response as immuno-therapeutic agents. Immunomodulation consists of two dimensions, one is to suppressing the immune response with medications like corticosteroids and various immunosuppressant drugs and the second dimension of immunomodulation is boosting the immune response by administrating immunostimulants which of the most ambient amiable and safest method (Elizabeth Devis, 2006). Immunomodulation is a procedure which can alter the immune system of an organism by interfering with its functions; if it results in an enhancement of immune reactions it is named as an immunostimulative drug which primarily implies stimulation of specific and non specific system, i.e., granulocytes, macrophages, complement, certain T-lymphocytes and different effector substances. Immuno-suppression implies mainly to
reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factor (Dashputre and Naikwade, 2010).

Not only medical science uses these immunostimulants to the lees but the utilisation of these natural supreme elements can also be upgraded to a wide spectrum of utilisation to the fields of Aquaculture (Citarasu et al., 2006), poultry and livestock (Thacker, 2010) bringing forth a colossal revolution in food production and meeting the needs of human beings. The combined use of immunostimulants with vaccines in poultry birds increase the efficacy of vaccines which protects them from variety of infectious agents under field conditions. Normally cytokines are used as immunostimulants along with avian vaccines which enhances their immuno response (Kumar et al., 2010). The need for aquatic animal vaccines, bacterins and other immunostimulants for preventing (or at least minimising the effects of) disease and / or infection is rapidly increasing. Tracing induced changes in fish by the use of immunostimulants, vaccines or environmental stressors (such as aquatic pollutants) is an important part of present day aquaculture (Anderson, 1992). The use of immunostimulants as dietary supplements can improve the innate defense of animals providing resistance to pathogens during periods of high stress, such as grading, reproduction, migration etc., (Ian Bricknell and Roy A. Dalmo, 2005). The use of immunostimulants as an alternative to the drugs and chemicals which unfortunately have adverse side effects or reverse phenomena, antibiotics which in some cases annihilates the intestinal commensial micro flora, is widely used in aquaculture practices to control fish diseases (Gelina et al., 2009). This tends to be an economic benefactor to the practice of aqua culture where there is high prone of diseases, and environmental stress factor avails resulting in completewipeing of fin fish and shell
fish stock commodities. The usage of immunostimulants in India is a wide spread practice in aquaculture especially in prawn and shrimp enterprise which tends to be an economic boon to this sector. There is a firm evidence showing, in order to increase the immunity of shrimps against White Spot Syndrome Virus (WSSV), the methanolic extracts of five different herbal medicinal plants like *Cyanodon dactylon, Aegle marmelos, Tinospora cordifolia, Picorhiza kurrooa* and *Eclipta alba* were used as a mixture and enormous survival of the shrimps were observed, showing better performance. This work brought a fine school of thought that the application of herbal immunostimulants will be effective against buffeting the viral growth and its adverse pathogenesis (Citarasu et al., 2006). Active immunisation is routinely used in aquaculture against vibriosis, furunculous, yersiniosis and bacterial kidney disease. When these vaccines were injected, immunostimulants can be easily added to heighten the immune response (Anderson, 2004). Complement activity was significantly increased in various fish groups treated with vaccines, immunostimulants like probiotics, tri-herbal and azadirachtin supplemented diets. Even the activity of lysozyme also enhanced increasing the disease resistance (Harikrishnan et al., 2009).

Some of the main immunostimulants used are levamisole (Siwiki et al., 1990, glucans (Jeney and Anderson 1993), chitins (Cuesta et al., 2003), and many others. It is well known that these agents stimulate the non-specific immune response (also called innate or natural protection) and boost the specific immune response. Therefore, immunostimulants can also be used as adjuvants to heighten the specific immune response. Glucans and other immunostimulants are now being added to fish feeds and vaccines. In some cases, they are injected alone at points of time near predictable fish
stressors. There is a firm evidence that the effectiveness of these agents in reducing mortalities. Immunostimulants are effective; the glucans are the most commonly used at this time. Indian traditional systems of medicines like Siddha and Ayurveda have suggested to increase the body’s natural resistance to disease. Recent screening with plants has revealed many compounds (e.g. alkaloids, flavonoids, quinones, terpenoids) with pronounced antioxidant, antineoplastic, antiulcer, anti-inflammatory and immunostimulating potential. So these age old medical practices evidently point out the indispensability of utilisation of immunostimulants (Dashputre and Naikwade, 2010).

Use of natural and herbal drugs in the treatments of ailments of hepatic disorders in Indian system of medicine is having long traditional history. At present, these natural agents are used as commercial available drugs containing ingredients of standardised quality. Ayurvedic system of medical practice clearly indicates the use of herbs extensively to treat chronic liver diseases and cirrhosis (Sanjiv Singh et al., 2010). The ayurvedic food supplements which boosts the body immune response against various pathogenic invaders are technically termed as “Rasayanas” (Chemicals). They also enhance the longevity of the individuals. When old mice were treated with varied doses of such immunostimulant rasayana known as Maharshi Amrit Kalash (MAK 5); it prevented in immunosenescence by suppressing the age associated glucose consumption of peritoneal macrophages and cellular immune function reduction (Inaba et al., 2005).

Immunostimulants can be well preferred as adaptogenic drugs because of their very fewer side effects and high virtuality and function. Immunostimulants are well utilised in the ancient but the most effective medical practice of Ayurveda as adaptogenic drugs even to now a day’s bringing forth excellent changes in health scenario.
Adaptogenic drugs that help an organism to cope better during stress and retard aging process are well recognised in Ayurvedic medicine. Herbal drugs are known to have immunomodulatory properties. These immunomodulatory agents act by stimulating both non-specific and specific immune responses. These are useful for prevention or treatment of immunodeficiency related disorders like AIDS. They are good adjuncts in therapeutic management of cancer. Many plants are claimed to have immunostimulant activity. Immuno-pharmacological properties of Picrorhiza kurroa, Ocimum sanctum were studied. Asparagus racemosus is an indigenous agent with immunostimulant properties, it showed a significant increase in the activity of macrophages. Immunomodulatory action is mostly concerned with cellular involvement of haemopoietic and lymphoid tissue. Medicinal plants are a rich source of substances which are claimed to induce a preferable immune response, to induce paraimmunity, the non-specific immunomodulation of essential granulocytes, macrophages, natural killer cells and complement functions. Because of the concerns about the side effects of the conventional medicine, the use of natural products to enhance the immune system, to boost up the immune response to heal various sorts of diseases and ailments use of medicinal plants as drugs, immunostimulants are therapeutic and safer alternatives for an effective treatment. These medicinal plants which serve as immunostimulants are having anti-oxidant, anti-carcinogrmic, anti-microbial, and immunomodulatory effects. Products have been employed in the development of immunomodulating agents, natural or synthetic to stimulate the natural defense mechanisms of the body and restore the original immunological functions. Such agents may act by increasing the specific immunity like humoral antibody response and cell mediated immune response (Rajendra Babu et al.,
Hence a new vista paved a way to use immunostimulants rather than the immunosuppressants.

The wide range of commercial immunostimulants are available these days for their unique use in various dimensions in life science applications like aquaculture, poultry and livestock and animal husbandry, apart from its wide spectrum utilisation in medical practices for human health prospective. Such a novel, commercial immunostimulant is the “Immunex DS” which is manufactured by PVS laboratories, Andhra Pradesh in India. This immunostimulant is highly engaged in high fidelity in aquaculture practices where there is enormous shrimp culture prevails. Immunex DS which is a complete amalgamation and a very special formulation consisting of Beta carotenes, L-lysine, DL methionine, Fatty acids, Livamisol hydrochloride, vitamins A, D3, E, C, B12, minerals like zinc, cobalt, manganese, selenium and probiotics lactobacillus, Saccharomyces cervisiae which are the vital immunostimulants individually deduced from natural, synthetic and organic in origin. Though this product is primarily used for shrimps, due to the constituents which it comprises of, it is selected as a vital asset of our research to implement in higher mammalian model such as Swiss albino mice (Mus musculus albicans) to endeavor and enrich the enigmatic enhancement of immune response.

Levamisole is a potent immunostimulant which stimulates macrophages and T-lymphocytes and improves cellular immunity by increasing secretion and proliferation of these cells (Ruiz-Morena et al., 1993; Fikret Demirci et al., 2005).

The implementation of immunostimulants in mice models is a wide discourse to amplify and analyse the immune response and immunocompetence experimental work in
these dimension vividly confirms that, when mice suffering with tumors were injected with intratumoural injection of immunostimulants to compare histological characteristics and immunogenicity to study the effect of human cutaneous melanomas (Salomon and Lynch, 1976). Various studies have been taken place to study the immunostimulative effects of natural immunostimulants and medicinal plants. An immunostimulant plant known as Achillea wilhelmsii, C. Koch (Asteraceae) which is a native of Iran known as “Boomadaran” is used to undertaken to study the immunomodulatory effect. Its aqueous extracts of varied doses was given to Swiss albino female mice. The results were quite promising. Plant extract at a dose of 100 mg/kg elicited a significant immuneresponse. This plant extract showed overall stimulative effect on both humoral and cellular immunity. This activity is the result of the presence of flavonoids or saponins, terpenoids and some alkaloids which augment the humoral response by stimulating the macrophages and B-Lymphocytes which are involved in anti body synthesis ((Fariba et al., 2009). Recent investigations gives a well established evidence that flavonoids act as immunostimulants by stimulating human peripheral blood leukocyte proliferation. They significantly increase the activity of helper T-cells, cytokines, interleukin-2, g-interferon, and macrophages and are there by useful in the treatment of several diseases by immune dysfunction. (Kawakita et al., 2005). The dried bark powder of Terminalia arjuna is a deciduous tree which has been known in Indian system of medicine to be beneficial for cardiac ailments and ischemic cardiac disease (Dwivedi, 1996). This powder in aqueous suspension were administered to male Swiss albino mice and male Wistar rats to study about anti-inflammatory potential against some phlogistic agents along with some immunomodulatory activity (Sumita Halder et al., 2009). Compound herbal medicinal
ingredients made up of astragalus poly saccharide, epimedieum poly saccharide, propolis flavones and gensenosides like immune potentiator were injected in rabbits after mixed with “Rabbit heamorrhagic disease” vaccine and then after 63 days challenged with RHD virus, and their immune response enhanced indicated by rabbit lymphocyte proliferation, gamma-IFN and IL-10 mRNA expression of T-Lymphocyte. These findings clearly indicate the immunostimulatory influence of these compound Chinese herbal medicinal ingredients (Yang et al., 2008). When BALB/c mice were treated with egg white solution, ovralbumin during experimental induced leishmaniasis, lesions induced by Leishmania were reduced by ingestion of ovalalbumin in sensitised mice. Lower parasitic load, and improved pathological outcome and enhanced immune response was observed (Saldahna et al., 2008).

The use of Immunex DS in a higher experimental mammal is a unique strategy of experimentation to study the effect of this product which is made of diverse elements, which are individually potent immunostimulants. It imparts the resistance power even in odd circumstances like severe stress, change in adaptability of the animal model, change in environment, severe induced pathogenesis as a result of artificial induction / infection of a disease factor such as an antigen or to higher assimilation and uptake of a vaccine to analyse the secondary immune response. The profounding impact of Immunex DS lies within its unique formulation, and its effect is multidimensional. This product mainly improves and enhances the strength, vigor and vitality of the animal model. It provides optimum growth and smooth maintenance of bodily system. It is constructive in better body’s defense mechanism yielding an enhanced immune response and lowers the susceptibility of various disease factors and infections. Immunex DS helps the animal to
relieve from stress and its unavoidable factors culminating from surrounding environment and rejuvenates the body and increases the natural stamina and boosts up the vital energy in the body. It increases appetite there by enhances the body weight of the animal. Immunex DS, as it acts like an adaptogenic drug prevents the colonisation of harmful bacteria in the gastro-intestinal tract and also hinders the accumulation of pathogens in it. It improves the sustenance and survival of the animal in adverse situations and during disease conditions. It improves the longevity of the animal model. The central theme of this work is based on the implementation of the Immunex DS in male Swiss albino mice (*Mus musculus albinus*), which are induced by a disease which is a burning prodigy of human health scenario, like Hepatitis B, and challenge its immunocompetence in the light of various morphological, physiological and biochemical prospectives.

Hepatitis B is a disease caused by hepatitis B virus (HBV) which infects the liver of hominoidae, including humans and causes an inflammation called hepatitis. Originally known as serum hepatitis, the disease has caused epidemics in Asia and Africa and it is endemic in China. Hepatitis implies the inflammation of the liver characterised by the presence of inflammatory cells in the tissue and organ. The term hepatitis is derived from ancient Greek language from two root words, *hepar* means the liver which is also known as the hepatic organ and *titis* meaning inflammation. A group of viruses known as the hepatitis viruses cause most of the liver damage worldwide. Hepatitis can also be due to the accumulation of toxins in the body, other infections or from autoimmune processes. They are various types of hepatitis viruses causing the disease and known as Hepatitis-A, Hepatitis-B, Hepatitis-C, Hepatitis-D, Hepatitis-E, Hepatitis-F and Hepatitis-G or also called as GBV-C (Ryder and Beckingham, 2001; Ganem and Schneider, 2001; Orito et
Studies in patients all around Asia brought out a shocking truth, that HBV genotype C is associated with a higher risk of developing cirrhosis than genotype B and preliminary data suggests and supports that genotype C, but not core promoter or precore stop mutations, correlates with more severe liver disease (Kao et al., 2000; Chan et al., 2002). Other clinical conditions have been implicated as predisposing factors for worsening of chronic hepatitis B including HDV, HCV or Human Immunodeficiency Virus (HIV) infection or concomitant alcohol consumption. Heavy consumption of alcoholic beverages increases the risk of progression of hepatitis to cirrhosis, resulting in the fibrous tissue in liver by six folds (Ikeda et al., 1998).

Other viral infections also can cause hepatitis such as mumps virus, rubella, cytomegalovirus, Epstein-Barr virus, yellow fever virus, and other herpes virus. Severe alcoholic consumption also causes hepatitis, which contains ethanol. A large number of drugs can cause hepatitis such as Agomelatine (anti-depressant), Amitriptyline, Azathioprine, hormonal contraceptives, Ibuprofen (NSAIDs), rifamycin (antibiotic for tuberculosis), Loratadine (antihistamine), Ketoconazole (antifungal), Methotrexate (Immunosuppressant), Nitrofurantoin (antibiotic) and over doses of Paracetamol and Zidovudine (antiretroviral drug against HIV). Troglitazone is an antidiabetic drug withdrawn in year 2000 for causing severe hepatitis in diabetic patients sometimes over utilisation of herbs and over dosage of nutritional supplements can cause hepatitis.

Many biological toxins are also responsible for the initiation of hepatitis in man, such as Amatoxin produced by mushrooms like Amanita phalloides (Death cap), Amanita orcreata (the destroying angel) and some species of Galerina. A portion of a single
mushroom can be enough to be lethal such as 10 mg of $\alpha$-amanitin. White phosphorous which is an industrial and war toxin also causes mutagenic hepatitis. Cylindrospermopsin, a toxin from the cyanobacterium *Cylindrospermopsis raciborskii* evokes hepatitis in humans. Anomalous presentation of Human Leukocyte Antigen (HLA) class II on the surface of hepatocytes, possibly due to the genetic predisposition or acute liver infection, causes a cell mediated immune response against the body’s own liver, resulting in a dreaded condition known as autoimmune hepatitis. Metabolic disorders, such as Hemochromatosis, the condition in which the extreme accumulation of iron in the body and Wilson’s disease which is due to accumulation of copper in the body triggers hepatitis resulting in tissue death (necrosis). The inadequate supply of blood to the hepatic organ causes Ischemic hepatitis (Shock liver disease). Hepatitis-B virus (HBV) chronically infects 300 million people world wide and it rapidly enhances the risk of developing hepatocellular carcinoma by a hundred fold (Beasley *et al.*, 1981; Lee, 1997; Maddrey, 2000; Milich and Liang, 2003; Tong *et al.*, 2005). Internationally accepted fact is males have a high prevalence and risk of Hepatitis-B infection (Beasley, 1988). Between 6-10 of every 100 young adult’s who have Hepatitis-B become chronic carriers. Hepatitis causes most of the human health problems and 0.5 to 1.2 million deaths are attributed to HBV infection annually. Hepatitis-B virus infection is the most common cause of hepatocellular carcinoma worldwide and ranks second only to cigarettes as the world’s leading cause of cancer. HBV infected persons have the highest potential to develop into hepatocellular carcinoma (Nakamoto *et al.*, 1998).

There is an immense need to control this human annihilator globally (Chen, 2010). In the past few years, significant advances have been made in molecular virology,
pathogenesis and treatment of HBV infection, however, there exists many challenges in HBV research, clinical management and exploration of safe and effective therapies for chronic Hepatitis-B. The earliest record of an epidemic caused by Hepatitis-B virus was made by Lurman in 1885 (Lurman, 1885), when an outbreak of small pox occurred in Bremen in the year 1883 and 1289; dock employees were vaccinated with crude lymph of other persons. After several weeks of this crude method of inoculation and unsophisticated immunisation technique resulted in death of 191 employees. And the other inoculated employees became severely ill with jaundice like symptoms and this malady was the foundational stone of the discovery of Hepatitis-B. At that time, this condition was diagnosed as Serum hepatitis. Later in the year 1909, similar out breaks of these were vividly reported following the introduction of hypodermic needles which were vigorously used and reused in unsterile, unhygienic and contaminated condition for the administration of Salvarsan for the treatment of syphilis. The virus was first discovered in 1965 by Baruch Blumberg in Australian aboriginal people hence it was first known as the “Australian antigen”; later it was known as the Hepatitis-B surface antigen (Alter and Blumberg, 1966). The complete discovery of the virus particle through electron microscopy was done by D.S. Dane and others in 1970 (Dane, 1970). By the early 1980’s, the genome of the virus was sequenced and the first vaccine was discovered and tested clinically (Galibert et al., 1979). The first Hepatitis-B vaccine was prepared from inactivated Hepatitis-B surface antigen particles purified from plasma of asymptomatic carriers of the same. Now-a-days, these vaccines take a new and most profounding role as therapeutic vaccines (Michel and Tiollais, 2010). Many therapeutic methods are utilised now-a-days to reduce the disease progression of HBV infection such as alanine
aminotransferase normalisation, Hepatitis-B virus suppression, HBeAg seroconversion, and histological improvement (Sato and Mori, 2010).

The virus was first discovered as “Australia antigen”, later named HbsAg for Hepatitis-B surface antigen in a victim’s blood (Blumberg et al., 1965). Considering the very short generation time for a virus, and the high error rate associated with the reverse transcription mechanism of this virus replication, makes decades of its discovery equivalent to million years of human evolution. In United States itself, the estimated toll victims of Hepatitis-B is one million (Wilkins et al., 2010). The carrier rate of hepatitis-B in India in different regions of the country is often quoted as being 4.7% (Ashish et al., 2007). The chronic carriers constitute the main human reserviour of hepatitis-B virus (Dienstag et al., 1991). This is the weighted mean of various studies and analysis of high risk populations like patients suffering from sexually transmitted diseases, thalassemia patients, professional blood donors, sex workers, drug abusers, dialyses patients, and medical staff and health care workers who are daily exposed to blood and other body fluids, which was undertaken by the Indian Medical Association (IMA), subcommittee on immunisation to evaluate the true prevalence of hepatitis-B in India (Lodha et al., 2001, Lodha and Kabra, 2001).

As of recent global estimates from 2004 onwards there are 350 million HBV infected persons are present, the prevalence is highly variable (Lee, 1997). Routes of infections are classified into three categories, vertical transmission consists of transmission of virus from mother to child through child birth. Early life horizontal transmission includes bites, lesions and sanitary habits, and the adult horizontal
transmission includes sexual intercourse, intravenous drug use etc. In China and South East Asia, the chief mode of the viral transfer is through the route of the vertical transmission i.e., through mother to child. Many pregnant mothers with chronic hepatitis-B are unaware of their infection and end up silently passing the virus to the next generation. The offsprings of such infected woman carriers may develop life long hepatitis B virus infection and develop liver failure or liver cancer in their later life.

Approximately 3% to 5% of infants born in Western Pacific Region (which includes Asia, the Pacific Islands and Australia) will acquire chronic hepatitis-B infection at birth if not immunised immediately after delivery. Each year, 8,69,494 infants will develop lifelong (chronic) hepatitis B infection in this region alone. Preventing infections acquired at birth and in early childhood is critical. Children have a 90% chance of becoming chronic carriers if infected at the time of birth and a 30% chance of becoming chronic carriers if infected between one and five years of age. Prenatally or childhood acquired HBV infection usually causes subclinical or anicteric acute hepatitis and is associated with a high risk of chronicity (40-90% of cases). Whereas adult acquired infection may cause acute symptomatic hepatitis (approximately 30% of patients) and is associated with a low risk of chronicity i.e., less than 5%. Chronic hepatitis B infection is a dynamic process with an early and fast and vast replicative phase and manifestation of an active liver disease and a late, low or non replicative phase with remission of liver disease. Perinatal acquired hepatitis-B infection is characterised by a prolonged immunotolerant phase with hepatitis-B e-antigen, HBeAg positivity, high levels of serum HBV and DNA, normal levels of aminotransferases, minimal hepatic damage, and very low rates of HBeAg spontaneous clearance. Patients with childhood or
adult acquired infection, and chronic hepatitis-B usually present in the immunoactive phase, with elevated and enhanced aminotransferases and high toxicity of liver and clear manifestation of necro-inflammation at examination at the histological level (Fattovich et al., 1997).

Transmission of hepatitis-B virus occurs via infectious blood and body fluids. Hepatitis-B is highly infective and rapidly transferred in high acceleration, if there is contact with infected blood or serum through puncture, wounds, splashes into the mucous membranes, contamination of the open wounds and injecting illegal drugs. It can be transmitted by sexual intercourse, during parturition. Usage of razors and needles of the victims in any purpose also causes the incidence of this disease. Even sharing the medical equipment in non-sterile environment also develops the enhancement of risk, the incidence of viral contamination in suction or accessory channels of gastro-intestinal endoscopes should be examined in order to get rid of this lethal condition.

The acute illness causes liver inflammation, vomiting, jaundice and chronic hepatitis-B may eventually leads to liver cancer, cirrhosis, which responds insensitive to present chemotherapy. Chronic infection with hepatitis-B virus is an important cause of cirrhosis and cancer of the liver, which is recently classified into eight genotypes, A-H. Accumulated evidence shows that the genotype influences both the clinical course of infection and the response to the treatment (Malmstrom, 2010). Acute infection with hepatitis-B virus is an ill condition that begins with general ill-health, loss of appetite, nausea, vomiting, tiredness, severe asthenia, pains in muscles and joints, arthalgia, dark urine, skin rashes, body aches, mild fever, dark urine, and progress to severe jaundice,
gall bladder obstruction and bile obstruction, fever leads to weakness, tiredness, inability to work for weeks or months and severe abdominal pain. Also, the skin becomes yellow in colour and itchy. Chronic infection with hepatitis B virus may be either asymptomatic or may be associated with a chronic inflammation of the liver, leading to cirrhosis over a period of several years. This type of severe inflammation dramatically enhances the risk of hepatocellular carcinoma (liver cancer) with a decrease in complete antioxidant activity and heavy raise in oxidative stress and enzymatic enhancement of ALK, s-GPT, s-GOT and LDH in (Mastoi et al., 2010; Kumada et al., 2010). Hepatitis-B incidence also shows its impact on the excretory organs where the kidneys become pale, necrotic, inflamed and development of an unique pathological condition known as Membranous glomerulonephritis (MGN) (Zhang et al., 2010). According to these studies the glomerulonephritis condition is severe in infants suffering due to chronic hepatitis where there is no use of antiviral treatment or the use of corticosteroid therapy to decrease the viral load and decrease proteinuria resulting from it. Nearly 95% of infected adults recover after few months. They clear the infection from their bodies and become immune. Unfortunately, about 5% of adults and 90% of infants and children under the age five are vigorously susceptible and unable to clear the viral load and become chronic sufferers. Persons who do not recover from hepatitis-B infection become chronically infected and they are the efficient carriers. Chronically infected persons are significantly at higher risk than the general population for liver failure or liver cancer.

The hepatitis-B virus primarily interferes with the functions of the liver by replicating in the hepatocytes. The extent of damage to the liver (the prime hepatic target organ) has been shown to correlate with the presence or absence of HBeAg in serum
HBV infection of adult is usually transient with the development of neutralising antibodies and immunity to re-infection. However, other cases resulted in prolong HBV infection that was accompanied by moderately severe chronic hepatitis and interface hepatitis. In humans, HBV infection of neonates results in chronic and persistent infection due to the underdeveloped immune system. In man with persistent HBV infection, liver damage is often observed ranging from mild chronic persistent hepatitis to chronic active hepatitis, cirrhosis, and hepatocellular carcinoma. Hepatocellular Carcinoma (HCC) is one of the most common types of cancer affecting Asians, particularly Chinese and Indians, and highly associated with Hepatitis-B infection (Okuda, 1986; Wong and Goh, 2006). Hepatocellular carcinoma is regarded as the fifth most common type of cancer occurring in the world (Freeman, 2002). The record of death toll held by HBV-related liver disease and hepatocellular carcinoma is 6,00,000 deaths worldwide annually (Borkakoty et al., 2008).

Hepatocellular carcinoma is a late complication of chronic Hepatitis-B infection that usually occurs at the fourth or fifth decade of life especially when the patients are older or they have developed severe liver cirrhosis. Chronic infection with hepatitis-B virus infections also enhance the chance of occurrence of Chronic Kidney Disease (CKD). There is a firm evidence suggesting that chronic hepatitis-B patients develop chronic kidney disease by receiving multiple medications resulting in drug interactions in a negative way leading into pathogenesis of drug-induced kidney disease (Fabrizi et al., 2010).

Hepatitis B virus (HBV) is not directly hepatotoxic but its interaction with the host immune system creates opportunity for the viral DNA integration into the host
genome. HBV causes the liver injury by an immune response against the virus infected liver cells (hepatocytes), although immunosuppression appears to enhance replication and lead to induce cytotoxicity. The interplay of the host immune response and the viral strength to replicate is a prime determinant of the likelihood of liver injury; its intensity and progression leading to hepatic cirrhosis. Viral mutations in the polymerase or the core gene affect replication and may enhance liver injury (Schoedel et al., 1989). It’s a bewildering fact that in patients with severe chronic hepatitis, and persistent replication of the virus leads to the high risk of liver cirrhosis (Borzi et al., 1992).

Numerous attempts were made to transfer the Hepatitis-B virus into a variety of experimental animals, some of them unsuccessful and some accomplished a form of hepatitis in mice. There is an immense need for the development of animal models for Hepatitis B virus infection for the crucial understanding concerning the rapid replication of virus, decease diagnosis, disease pathogenesis and invention and improval of specific drugs (Zhan Gao et al., 2010). But in fact, the major obstacle and hinderance there lies with; a easy-to use conventional animal model has not been detected for in vitro cell model for the production of antihepatitis drugs (Guha et al., 2004; Jia-Ming Chang et al., 2010). Now-a-day extensive efforts have been put forth in developing of an effective and efficient animal model for the complete study of this human ailment (Sitia et al., 2007; Chayama et al., 2011). Due to the lack of a specific animal model; there is a great gulf to know the immunopathogenic proces from hepatitis B virus infection and its genesis into liver fibrosis and liver carcinoma (Jin et al., 2011). The factors and determinants of the immune response to the Human hepatitis virus are scarcely understood as experimental studies in Man and Chimpanzees were done with some prevailing limitations. So, the
selection of the mice model to induce HBV is the appropriate strategy to analyse the cellular and humoral responses (John von Freyend et al., 2010). The viral agent causing acute hepatitis was encountered in weanling mice of Princeton strain, which invades and inhabits in liver, spleen, kidneys, heart, blood, urine and also in intestinal contents (John B. Nelson, 1952). Human Hepatitis Virus (HBV), as well as a good many number of other viruses may infect other mammalian hosts such as ground squirrels, arctic squirrels, woolly monkeys and mice. The woodchuck hepatitis virus (WHV) has similar characteristics with the Human hepatitis-B virus (HBV), and provides an excellent animal model to study the pathogenesis of hepatocellular carcinoma. In wild animals infected with woodchuck virus has been shown to develop hepatitis and liver cancer (Tennant et al., 2004; Tang and Mc Lachlan, 2002). In experimental conditions an enhanced, almost 100% development of hepatocellular carcinoma was reported in the experimental animals, mostly mice. The experimental findings revealed that there was a strong bond of integration of wood chuck hepatitis viral genome DNA with DNA of the hepatocyte (Synder et al., 1982).

HBV infection was developed in a mouse model (C57BL/6) by hydrodynamic transfection, and then they were injected intra peritoneally triple with 0.2 ml of dexamethason, every two days before HBV infection. The outcome was the cent per cent positiveness for HBsAg and HBeAg in serum and liver. The viral load was significantly higher than the controls on day 10, 30 and 60 after HBV transfection. By suppression of the immune response of mice injected with dexamethosone, the manifestation of these antigens for an extended longer time, these mouse models could be highly used in development of HBV vaccine (Guo et al., 2010).
The nucleocapsid encloses the viral DNA and a DNA polymerase which does the function of reverse transcription. This virus is 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 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 (Howard, 1986). Humans with persistent hepatitis-B virus infection have tend to contain high levels of viral replication in above 95% of the hepatocytes in the liver, and up to millions of viral particles and non-infectious surface antigen particles in serum (Nassal and Schaller, 1996). During the infection period, filamentous forms of surface antigen containing particles are produced. Also circulating in the blood of infected humans is a secreted form of the viral core antigen, the so-called “e-Antigen” (HBeAg) which is thought to play a pivotal role in maintaining the persistence infection. The life cycle of hepatitis-B virus is highly complex, which uses reverse transcription as a part of its replication process. The virus gains access into the living cells by bounding itself to an unknown receptor on the cells surface and penetrates by the phenomena of endocytosis. Because the virus multiplies via RNA made by the host enzyme, the viral genomic DNA has to be transferred to the cell nucleus by host proteins called as chaperones. Hepatitis-B virus genome replication requires the whole entity of viral factors such as pregenomic RNA and polymerase and also host factors like heat shock proteins and protein kinase C. many reports vividly suggest that there are several unidentified host factors which promote encapsidation. Recent research brought an interesting key role of a new host factor, nucleophosmin (B23) that interacts with HBV core protein 149 (Cp149). The
partially double stranded viral DNA is then mutated into double stranded and transformed into covalently closed circular DNA that serves as a template for transcription of four viral mRNAs. The largest mRNA, which is longer than the viral genome is used to make the new copies of the genome and to make the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions which are released from the cell or returned to the nucleus and recycled to produce even more copies (Beck and Nassal, 2007; Bruss, 2007). The long mRNA is then transported back to the cytoplasm where the Virion protein synthesises DNA via reverse transcriptase activity. Viral mutations in the polymerase or the core gene effect replication and may enhance liver injury (Bruss, 2004). During HBV infection, the host immune response causes both cellular damages of hepatocytes 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 liver injury associated with hepatitis-B virus infection. By killing infected cells and by producing antiviral cytokines capable of purging HBV from viable hepatocytes, these cytotoxic T-lymphocytes eliminate the virus. Although the damage of the hepatic organ undergoes severe damage, which is initiated and mediated by CTLs, antigen-nonspecific inflammatory cells can worsen CTL-induced immunopathology, and the platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver. The pathogenetic and antiviral potential of the cytotoxic T-lymphocyte response to hepatitis-B virus has been proven by the induction of a severe necroinflammatory liver disease following the adoptive transfer of HbsAg specific CTL into HBV transgenic mice (Chisari et al., 2010).
It has been postulated that in the persistently infected individuals the HBV specific immune response is too weak to eliminate the virus from all the infected liver hepatocytes, but sufficiently strong to continuously annihilate HBV infected hepatocytes and to induce a chronic inflammatory liver disease. Furthermore, a clear correlation was demonstrated between increased level of serum HBV DNA and acute exacerbation of liver injury in patients with chronic hepatitis B which is triggered by the change of viremia and HBV replication (Morell et al., 1983). The T-cell response during hepatitis-B in people is manifested by a vigorous, polyclonal and multispecific cytotoxic and helper T-cell response. Although clearance of viral infection is most widely thought to indicate the killing of the infected liver cell by virus specific T-cells, it was firmly suggested that non-cytolytic intracellular viral inactivation by cytokines released by virus inactivated lymphono-nuclear cells have a well defined role in the clearance of this virus without killing the infected cells (Sarzotti et al., 1996). Recent studies using a transgenic mouse model of hepatitis-B virus infection have also shown that adoptively transferred, virus specific cytotoxic T-cells can strongly abolish hepatitis-B virus gene expression and replication in the liver without killing the hepatocytes, which prevents hepatocellular carcinoma and further progression towards cirrhosis of liver (Guidotti et al., 1996). Additional factors that may contribute to viral persistence in the hepatocytes may include immunological tolerance to viral antigens, viral inhibition of antigen processing or presentation, infection of immunologically privileged sites, modulation of the response to cytotoxic mediators and viral mutations (Guidotti and Chisari, 1996).
In the past few decades, significant advances have been made in the disciplines of molecular virology, pathogenesis and prophylactic diagnosis and treatment of hepatitis-B, nevertheless there are still many challenges persisting to overcome concerning HBV research, clinical management, safe and effective therapies. Therefore, they should be taken an indispensable step globally to control Hepatitis-B infection treating the chronically infected persons and preventing the susceptible ones with immunoprophylaxis. The outstanding need is the universal vaccination against HBV infection regarding infants, adults and high risk groups comprising especially of sex workers and dialysis patients. There is an urgent need to develop new vaccines and new adjuvants to get ride of this sustaining menace (Bagnato et al., 2009). In these prominent risk groups which have a high priority of incidence and infection of hepatitis-B virus, vaccination should be administered which enhances the immune status of these persons and reduce the economic rip due to usage of prophylactic strategies. There is only one essential path to thread this engulfing mortality is by immunisation. The most efficient way to prevent this is to encourage universal vaccination in order to reduce the morbidity and mortality status caused by HBV (Marillia Dourado et al., 2004). For more than a decade, the recombinant hepatitis-B virus vaccine has been used. It consists of non-glycosylated HBsAg particles, in comparison with natural HBsAg. It is comparable in immunogenicity, protective efficiency, and safety to the first generation of vaccines which are derived from plasma (Alder and Bourgeois, 2001). But in fact a fewer percentage of healthy and highly immunocompetent adults (2-10%) do not show any response to vaccines due to the production of protective levels of anti-HBS antibody. This condition has many external and internal factors such as environmental factors, sex,
age and inherent genetic constitution (Van Loveren et al., 2001). This situation also clearly manifests a malfunctioning defect in either B or T-cell functions in unresponsive individuals.

Such a criterion made an outstanding foundational basis for initiating this research work by treating the male Swiss albino mice with an novel immunostimulant, Immunex DS and then inoculating the animal model with commercially available r-DNA vaccine employed for the Hepatitis immunisation commercially known to the world as Gene Vac B vaccine which contains the surface antigen HBsAg. The understanding of the immune response upon Hepatitis B is useful to develop appropriate therapeutic strategies to control this monstrous menace, as well as to improve current knowledge regarding hepatitis prognosis.

The present studies were undertaken to understand the level of immune response stimulated due to Immunex DS and inoculated with Gene Vac B vaccine with regard to certain biochemical changes. From our pilot experimental study, it is known that a dose of 150 mg/mouse Immunex DS is the immunologically suitable dose to evoke the immune response in swiss albino mouse model. Also, the present study is useful to analyse the effect of Gene Vac B vaccine in Immunex DS treated mice. The present work is designed to determine the following:

1) Estimation of total proteins and free aminoacids, estimation and extraction of DNA from liver and spleen and activity of liver SOD in mice treated with a single dose of Immunex DS (150 mg/mouse) for one day and inoculated with a single dose of 0.07 ml of Gene Vac B vaccine (on day 7).
2) Estimation of total proteins and free aminoacids, estimation and extraction of DNA from liver and spleen and activity of liver SOD in mice treated with a single dose of Immunex DS (150 mg/mouse) for one day and inoculated with a single dose of 0.1 ml of Gene Vac B vaccine (on day 7).

3) Estimation of total proteins and free aminoacids, estimation and extraction of DNA from liver and spleen and activity of liver SOD in mice treated with a single dose of Immunex DS (150 mg/mouse) for one day and inoculated with a single dose of 0.2 ml of Gene Vac B vaccine (on day 7).

4) Estimation of total proteins and free aminoacids, estimation and extraction of DNA from liver and spleen and activity of liver SOD in mice treated with a single dose of Immunex DS (150 mg/mouse) for one day and inoculated with a single dose of 0.4 ml of Gene Vac B vaccine (on day 7).

5) Estimation of total proteins and free aminoacids, estimation and extraction of DNA from liver and spleen and activity of liver SOD in mice treated with a single dose of Immunex DS (150 mg/mouse) for one day and inoculated with a single dose of 0.8 ml of Gene Vac B vaccine (on day 7).

6) Estimation of total proteins and free aminoacids, estimation and extraction of DNA from liver and spleen and activity of liver SOD in mice treated with a single dose of Immunex DS (150 mg/mouse) for one day and inoculated with a single dose of 1 ml of Gene Vac B vaccine (on day 7).
7) Estimation of total proteins and free aminoacids, estimation and extraction of DNA from liver and spleen and activity of liver SOD in mice treated with repeated doses of Immunex DS (75+75 mg/mouse) and inoculated with a single dose of 0.8 ml of Gene Vac B vaccine.

8) Estimation of total proteins and free aminoacids, estimation and extraction of DNA from liver and spleen and activity of liver SOD in mice treated with repeated doses of Immunex DS (75+75 mg/mouse) and inoculated with a single dose of 1 ml of Gene Vac B vaccine.

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