Chapter- 1

INTRODUCTION AND REVIEW OF LITERATURE

Hepatitis B is an infection illness 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 (Baker et al., 1996). Chronic hepatitis B, a serious liver disease caused by hepatitis B virus, is one of the most prevalent yet most neglected health problems in the world. It affects nearly 1 in 20 (approximately 350 million) people worldwide and causes 60-80% of the global burden of hepatocellular carcinoma (HCC) (primary liver cancer) (Hwang et al., 1996; Parkin, 2006). Hepatitis B is among the top three causes of cancer death worldwide (Parkin et al., 2005). The disease has caused epidemics in parts of Asia and Africa, and it is endemic in China (Williams, 2006). The most common type of cancer which is highly associated with hepatitis B infection is reported in Asians, particularly in Chinese and Indians (Okuda, 1986; Wong and Goh, 2006). HBV related liver disease and HCC deaths have been found to be 6,00,000 worldwide annually (Borkakoty et al., 2008).

In India, the national HBV prevalence rate has been estimated to be 4% with approximately 36 million carriers overall (Tandon et al., 1996). An extensive review by the Indian National Association for the Study of Liver Diseases estimated the average national prevalence rate as 4.7% (Lodha et al., 2001). However, as with other countries that cover a large geographic area, the prevalence of hepatitis B is variable throughout the country with a gradient of generally increasing from north to south. The lowest prevalence is 2.3% in a large cohort of 20,000 blood donors in Northern India (Behal et al., 2008). The highest reported rate is 5.7% in a community based study where in almost 2000 people from Southern India (Kurien et al., 2005).
Most of the hepatitis B deaths (94%) were attributed to complications of chronic infection, such as cirrhosis and HCC, and only 6% were attributed directly to acute hepatitis B (Goldstein et al., 2005). About a third of the world’s population, more than 2 billion people have been infected with the HBV (WHO, 2009). This includes 350 million chronic carriers of the virus (Lavanchy, 2004). Hepatitis B is a Hepadnavirus - hepa from hepatotrophic and dna because it is a DNA virus (Zuckerman, 1996). It is a double stranded DNA virus which acts as a template for transcription of several viral mRNAs (Seeger and Mason, 2000). HBV is a DNA virus with human-only reservoir, a worldwide health problem (Hwang and Cheung, 2011). HBV infection is estimated to be the cause of 30% of cirrhosis and 53% of liver cancer in the world (Perz et al., 2006). The virus replicates through RNA intermediate form by reverse transcription in this respect they are similar to retroviruses (Locarnini, 2004). HBV has been linked to the development of Membranous glomerulonephritis (MGN) (Lai et al., 1991).

Possible forms of transmission includes unprotected sexual contact, blood transfusion, re-use of contaminated needles and syringes, and vertical transmission from mother to child during child birth (Hoofnagle, 1981; Kew, 1981). HBV can also been transmitted between family members within households, possibly by contact of non intact skin or mucous membrane with secretions or saliva containing HBV (Petersen et al., 1976). Patients with HIV infection may lodge high levels of HBV DNA and hepatic necroinflammation with anti HBC but not HBsAg so called “occult HBV” (Soriano et al., 2005). Progression from acute to chronic HBV infection is influenced by patient’s age. At acquisition of the virus usually lifelong infection is established in more than 90% of infected persons. In Asian countries, there is 90% prevalence of HBV infection (Hoofnagle et al., 2007; Dienstag and Isselbacher,
Individuals who remain HBsAg positive for at least six months are considered to be Hepatitis B carriers (Lok and McMahon, 2007). Upper limits of normal Alanine transaminase (ALT) and Aspartate transaminase (AST) should be 30 U/I for men and 19 U/I for women (Prati et al., 2002).

Decreased antioxidant enzyme activities are associated with severe liver injury and hepatocarcinogenesis in mouse models (Elchuri et al., 2005). The immune response initiated by the T-cell response to viral antigens is thought to be fundamental for viral clearance and disease pathogenesis in HBV infection. The T-cell response is characterized by vigorous, polyclonal, and multispecific cytotoxic and helper-T-cell response. In chronic carriers, the immune response is not able to eliminate the virus, as it is weak or undetectable. Thus a dominant cause of viral persistence could be the existence of a weak antiviral immune response. The central role of cellular immunity in disease pathogenesis, strategies have been proposed to manipulate this cellular immune response in favour of protection from disease (Yano, 2002).

HBV infection is a major worldwide health problem, and chronically infected individuals are at high risk for developing cirrhosis and hepatocellular carcinoma; despite the availability of an HBV vaccine, more than 350 million people were chronically infected worldwide, and the few treatments currently available have a limited rate of efficacy (Blumberg, 1997; Rogler, 1991). The narrow host range of HBV and the lack of both in vitro systems and the convenient animal models have greatly hampered the understanding of the complete virus life cycle, as well as the development of more effective antiviral drugs aimed at eradicating the virus from chronic carriers (Ganem, 1996). Chimpanzees are the only animal species infectable with HBV (Guidotti et al., 1999; Ogata et al., 1999) but studies with these animals and evaluation of antiviral therapies are severely restricted because of their limited
availability and high costs. HBV-related hepadnaviruses such as woodchuck and Pekin duck hepatitis B viruses are often used for assessment of antiviral drugs (Tennant, 1994; Mason et al., 1998; Luscombe et al., 1996) and have provided important information about factors involved in establishment of virus infection, viral persistence, and hepatocarcinogenesis (Breiner et al., 1998; Chen et al., 1993; Dandri et al., 1996; Fourel et al., 1990; Bruns et al., 1998; Jilbert et al., 1992). However, woodchucks are relatively large animals of outbred origins that are difficult to handle in many laboratories. The development of HBV-expressing transgenic mice has also been provided important insights regarding viral pathobiology and the role of HBC gene products in hepatocellular injury (Fourel et al., 1990; Chisari and Ferrari, 1995; Guidotti et al., 1996; Kim et al., 1991; Guidotti et al., 1994; Chisari et al., 1987).

Serum concentrations of total protein, albumin, bilirubin, and transaminases were similar in uPA/RAG-2 mice containing human hepatocytes compared with control nontransplanted littermates (Dandri et al., 2001).

Mice fed with the transgenic lupin tissue developed significant levels of hepatitis B virus-specific antibodies. Human volunteers, fed with a plant derived edible vaccine (protein) developed specific serum-IgG response (Kapusta et al., 1999). In alcohol-induced liver disease, acute viral hepatitis, and chronic hepatitis, silymarin, glycyrrhizin are thought to normalize the serum transaminase levels in patients with chronic hepatitis (Ferenci et al., 1989; Yoshioka et al., 1989; Marinos et al., 1995). It has been shown to protect against glutathione depletion (Mira et al., 1994) and iron overload (Pietrangelo et al., 1995) in rats and against lipid peroxidation (antioxidant activity) in rat hepatocytes (Carini et al., 1992) and increase of protein synthesis through stimulation of rRNA polymerase in hepatocytes (Sonnenbichler and Zetl, 1986). Plants of the genus *Phyllanthus* are found in most
tropical and subtropical areas. They have been used as an aqueous extract in folk medicine mainly in India and China to treat diabetes, kidney and urinary problems, diarrhea, and hepatitis B. The mechanism of action of *P. amarus* appears to be related to inhibition of HBV polymerase activity (Blumberg *et al*., 1990). Studies conducted in human cell lines susceptible to hepatitis B infection, *P. amarus* inhibited HBV polymerase activity, and in hepatitis B transgenic mice it decreased HBV mRNA transcription, therefore suggesting that *P. amarus* can suppress viral replication (Ott *et al*., 1997; Lee *et al*., 1996). In human hepatoma cells, *P. amarus* suppressed Hepatitis B surface antigen (HBsAg) gene expression and HBsAg production, but did not inhibit hepatitis B e antigen production (Yeh *et al*., 1993).

Gene Vac-B™ (Recombinant Hepatitis-B Vaccine, I. P.) is a non infectious recombinant DNA Hepatitis B vaccine. It contains purified surface antigen of the virus obtained by culturing genetically-engineered *Hansenula polymorpha* yeast cells having the surface antigen gene of Hepatitis B virus. The HBsAg expressed in the cells of *Hansenula polymorpha* is purified through several chemical steps and formulated as a suspension of the antigen adsorbed on aluminium hydroxide and thiomersal is added as preservative. The vaccine does not contain any material of human or animal origin. Gene Vac-B™ is indicated for active immunization against Hepatitis-B infection in subjects considered at risk of exposure to HBV-positive material. Immunization against hepatitis B is expected in the long term to reduce not only the incidence of this disease, but also its chronic complications such as chronic active hepatitis B and hepatitis B associated cirrhosis and primary HCC. In areas of low prevalence of hepatitis B, immunization with Gene Vac-B™ is recommended for neonates/infants and adolescents as well as for subjects who are, or will be, at increased risk of infection. Gene Vac-B™ should be injected intramuscularly in the
deltoid region in adults and children or in the anterolateral thigh in neonates, infants and young children. The worldwide importance of human HBV infection and the toll it takes in chronic liver diseases, cirrhosis and hepatocarcinoma, make it imperative that a vaccine be developed for worldwide application (Deinhardt and Gust, 1982). Human hepatitis B vaccines are presently prepared using hepatitis B surface antigen (HBsAg) that is purified from the plasma of human carriers of HBV infection (Buynak et al., 1976; Adamowicz et al., 1981; Coutinho et al., 1983).

Hepatitis B vaccine is of yeast origin; HBsAg of subtype adw was produced in recombinant yeast cell culture, and the purified antigen in alum formulation stimulated production of antibody in mice, grivet monkeys and chimpanzees. Vaccinated chimpanzees were totally protected when challenged intravenously with either homologous or heterologous subtype adr and ayw virus of human serum source which is the first example of vaccine produced from recombinant cells which is effective against a human viral infection (McAleer et al., 1984). During the last two decades very intensive investigations are carried out on the preparation, experimental and clinical characteristics of one relatively new category biologically active substances so called immunostimulants. They are the products from natural or synthetic origin with different chemical characteristics and mechanism of action (Klausen et al., 1991; Persson et al., 1994; Petrunov et al., 1991; Petrunov, 2004).

Immunostimulants activate different elements and mechanisms of the humans and animals immune systems, they reinforce body’s natural resistance in order to successful cope up with various viral and bacterial infections, they stimulate the main factors of the immune system like phagocytosis, properdin and complement systems, protective secretory IgA antibodies, α- and γ-interferon release, T and B lymphocytes, synthesis of specific antibodies and cytokines, pulmonary surfactant. Nowadays not
less than 10-15% of the human beings (immunocompromised persons) having
damaged immune system are easily exposed to common infectious diseases.

In Bulgaria for more than 20 years polybacterial immunostimulants (by oral
administration) intended to stimulate the natural immune system and to help in
recovering and in prevention of the infections of respiratory and urinary systems, oral
cavity and periodontal. Bacterial species entering the immune system stimulate the
synthesis of homologous specific protective IgG, IgA and IgM antibodies. That means
polybacterial immunostimulants act also like bacterial vaccines (Petrunov et al.,
2007). According to Sakai, (1999), immunostimulants can be divided into several
groups depending on their sources; bacterial, algae-derived, animal-derived,
nutritional factors, and hormones/cytokines. An immunostimulant is a naturally
occurring compound that modulates the immune system by increasing the host’s
resistance against diseases caused by pathogens (Peddie et al., 2002).

The use of immunostimulants in vaccine formulations has given very good
antibody responses (deBaulny et al., 1996; Anderson, 1997; Figueras et al., 1998;
Kawakami et al., 1998; Romalde et al., 1999). The biological effects of
immunostimulants are highly dependent on the receptors on the target cells
recognizing them as potential high risk molecules and triggering defense pathways.
However, many mammalian receptors bind immunostimulants. Nevertheless,
assuming fish and mammalian cells contain many similar receptors (Bricknell and
Dalmo, 2005). Immunostimulants are often naturally occurring molecules that can be
obtained from a natural source in large quantities, they can improve the innate defense
of the animal providing resistance to pathogens during periods of high stress, such as
grading, sea transfer and vaccination (Robertsen, 1999; Conceicao et al., 2004; Bagni
et al., 2000; Efthimiou, 1996; Kennedy et al., 1998). Innate defense may be induced
by an increase of known defensive proteins such as complement or interferon or the activation of cellular defenses such as macrophages (Sakai, 1999). Propagermanium (PPG) is thought to inhibit hepatitis B virus replication and eliminate it from the system through immunostimulation and protection from viral infection by increasing the interferon (IFN) production at the infected area (Yano, 1994). Prolonged administration of a commercial β-glucan based immunostimulant preparation EcoActiva™ during winter may enhance macrophage function and growth rates at the time of increased disease susceptibility and little or no growth (Cook et al., 2003).

Muscle tissue in mammals may be divided into three types: skeletal (striated) muscles- used for movement of organs etc, visceral (smooth) muscles - found in the walls of the digestive tract, arteries, veins, uterus, bladder and many glands and cardiac (heart) muscles - a special type of muscle found only in the heart (Campbell, 2007). Skeletal muscles, visceral muscles and cardiac muscles are connected by many intermediate forms. The visceral and cardiac muscles contract independently of voluntary control, while the skeletal muscles are subject to voluntary control.

When a bit of fresh skeletal muscle is examined under the microscope, the muscles cells (i.e muscle fibers) appear as long, spindle shaped bodies which are thickened in the middle and become narrow towards their pointed ends. In many places in the body but particularly in the skin, striated muscle fibers are arranged parallel to one another in one plane. The muscle fibers are large and multinucleated cells. Most skeletal muscles move bones and cartilages, some also cause movement of soft parts, for example, facial muscles. Skeletal muscle is influenced directly by the central nervous system and is under the control of the will.

The muscle fiber (large cell) is multinucleate i.e. it is a syncytium. The fibers are unbranched, each fiber is enclosed in a thin membrane, the sarcolemma, which is
a specialized cell membrane. The protoplasm of the fiber, sarcoplasm, contains five longitudinal myofibrils which extend throughout. Electron micrographs show that each myofibril is composed of two types of short myofilaments which are precisely arranged giving the appearance of transverse banding, the striations. The abdominal muscles are important to provide cardiac and respiratory support when the diaphragm muscles have been damaged by any adverse situations. Muscular dystrophy may lead to decreased function of various muscle group-specific muscles involved in respiration causing breathing difficulties as well as leading to cardiac problems.

Physiological and histochemical studies on muscles of NMRI mice showed increased fatigue-resistance and increase in oxidative enzyme activity; these are the favorable adaptations of muscles to endurance exercise (Gollnick and King 1969; Baldwin et al., 1972; Gollnick et al., 1972; Saltin et al., 1977; Edgerton, 1978; Dudley et al., 1982; Green et al., 1983; Howald et al., 1985). Split muscle fibers are generally associated with pathological states (Bosanquet et al., 1973). Apart from myopathies (Schmalbruch 1976; Swash et al., 1978; Carpenter and Karpati, 1984), split muscle fibers can be found after experimental muscle damage (Hall-Craggs and Lawrence, 1970; Hall-Craggs, 1972), synergist incapacitation (Van Linge, 1962; (Hall-Craggs, 1970, 1972; James, 1973; Vaughan and Goldspink, 1979; Atherton et al., 1981), muscle stretch (Sola et al., 1973) and denervation (Miledi and Slater, 1969; Yellin, 1974; Carraro et al., 1985). Whereas all these circumstances can be referred to either as truly pathological and highly unphysiological, split fibers have also been found after exercises such as weight lifting (Gonyea et al., 1977). After enforced endurance exercise in mice (Silberman et al., 1983) and in rats (Elder and Vassallo, 1986), fiber splitting has been noticed. Split fibers can form as a consequence of muscle injury. In humans and in animals, degeneration, necrosis and regeneration of
muscle fibers can occur after strenuous overuse of muscles (Highman and Altland, 1963; Greenberg and Arneson, 1967; Geller, 1973; Bartsch et al., 1977; Vihko et al., 1978, 1979; Armstrong et al., 1983; Hikida et al., 1983; Jones et al., 1986) and enforced moderate exercise (Kuipers et al., 1983).

Reduced tolerance to exercise can result from inherited factors such as a glycogenolytic defect in the muscle (Schmid and Mahler, 1959). Impairment of muscle function because of overuse is known, although not many are aware of the more severe consequences of exercise-induced muscle injury. With respect to military training, exertional rhabdomyolysis, with myoglobinuria can cause acute renal failure (Howenstein, 1960; Hamilton et al., 1972; Geller, 1973) sometimes followed by death (O’Donnell, 1971). Although it is possible for a muscle fiber to change its properties as a result of a new activity pattern (Pette and Vrbova, 1985), evidence has been found that, at least in part, fiber type transformation in low-frequency stimulated fast-twitch rabbit muscles results from necrosis and replacement of fibers (Maier et al., 1986). Split fibers and muscle-fiber necrosis are known from pathological and physiological conditions (Irintchev and Wernig, 1987).

Studies on contents of glycolytic and energy intermediates in muscle in vivo indicates that such data are available only for tissues of rat, mouse, guinea pig and, in some cases, the frog (Williamson and Brosnan, 1974; Beis and Newsholme, 1975). Motamedi-Shad et al., (2009) studied the effect of different monosaccharide derivatives, featuring the main characteristics of heparin and heparin sulphate (HS) building blocks, on the aggregation kinetics of human muscle acylphosphatase (mAcP). The effect of heparin and HS on protein aggregation arises from the clustering and regular distribution of their composing units on a polymeric structure. Expression of nestin is generally ceased in mature cells but resumes following injuries
(Frisen et al., 1995; Yang et al., 1995). After shearing or in situ injuries, nestin reappears in myocytes of rat skeletal cells and its expression reaches maximum level at 3-5 days post injury, then become down regulated thereafter (Vaittinen et al., 2001). Use of herbal drugs is wide-spread in the control of hepatic diseases (Saleem et al., 2010). Although more than 300 preparations are available for the treatment of jaundice and chronic liver diseases in Indian Systems of Medicine, studies on P. amarus have confirmed that this plant preparation possessed anti-viral against hepatitis B and C viruses, hepatoprotective and immunomodulating effects, besides anti-inflammatory properties (Thyagarajan et al., 2002).

Oral administration of probiotic Lactobacillus casei either prior to or simultaneously with Giardia infection to malnourished mice led to significantly enhanced activity of disaccharidases compared with malnourished and Giardia-infected mice (Shukla et al., 2013). Worsen the health and continue to be an important cause of morbidity and stunting of growth among children in developing countries (Junqueira and Queiroz, 2002; Matos et al., 2008). More than 90% of the world’s stunted children live in Africa and Asia, where rates of stunting are 40% and 36% respectively (UNICEF, 2009).

In India, 50% of child deaths are due to malnutrition while 46% of children under five years of age in rural India and 33% in urban India are underweight, with 16% being severely undernourished and 48% stunted. Malnourished individuals are more susceptible to various diseases (Calder and Jackson, 2000). Nutritional interventions reduce the morbidity and mortality from gastrointestinal disease in malnourished individuals (Rice and Schaefer, 1981). Among various nutrient interventions, immunostimulants and/or probiotics (particularly lactic acid bacteria) are generally regarded as safe for their inhibitory and immunomodulatory activities,
and are defined as live microorganism are beneficial, safe, effective, and cheap to the host (FAO/WHO, 2001). Earlier, it was observed that the probiotic *L. casei* supplementation even to malnourished/renourished mice have been effective in reducing the severity, duration, and pathological alteration in *Giardia*-infected mice (Shukla et al., 2008, 2012; Shukla and Sidhu, 2011a).

Malnutrition and specific nutrient deficiencies are the leading cause of immunodeficiency diseases, and death in developing countries of the world (Prentice et al., 2008). Severity of infection was reduced due to the probiotic supplementation that is known to improve the anthropometric and biochemical parameters due to better colonization of healthy bacteria (Shukla et al., 2010; Tiwari et al., 2009; Shukla and Sidhu, 2011b; Mastronicola et al., 2011; Ding et al., 2007). Microbiota was improved which is essential for the maintenance and physiology of healthy bacteria as it synthesizes various substances (biotin, pantothenate, riboflavin, pyrodoxine, and vitamin K) important for metabolic processes and absorption of nutrients (Younes et al., 2001; Miyazawa et al., 1996). Increased mass of microbiota was found in small intestine of probiotic-treated malnourished mice (Shukla et al., 2013).

The decreased activity of mucosal disaccharidases and alkaline phosphatases was found in mice belonging to various malnourished groups (Sood et al., 1987; Gillon and Ferguson, 1984). The probiotic *L. casei* supplementation either before or simultaneously with *Giardia* infection to malnourished mice helped in restoring the intestinal mass and activity of the intestinal enzymes (Humen et al., 2005; Southcott et al., 2008; Shukla et al., 2013). The cells of all living organisms synthesize enzymes; they act like catalyst and accelerate the metabolic reactions.

All enzymes are proteins and their catalytic activities are depended on their folded polypeptide chains. Under pathological condition, even minor alterations in
their structure may result in the loss of activity. In living organisms enzymes are rapidly degraded in diseased state and their synthesis may be replenished during disease free state. Each enzyme is highly specific and requires specific substrate and majority of them carryout only one type of reaction. Microbial infections may denature the enzymes. Enzymes are not only the foundation of human life but also fundamental to health. Rajamanickam and Muthuswamy, (2008) stated that enzymes are necessary for the normal cellular metabolism. Liver enzymes fall into three categories like metabolic, digestive and food enzymes.

Enzymes are also classified according to catabolic reactions. The major six classes of enzymes are – oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. The enzymes present in plasma can be grouped as functional (plasma active) and non functional (exocrine secretions and intracellular enzymes). Functional enzymes perform physiological function in blood which include the proenzymes of blood coagulation, lipoprotein lipase etc. They are synthesized in the liver and are present in equivalent or higher concentration when compared to other tissues. Under normal conditions non functional enzymes are found in low concentration in serum but they may reach in high concentration during the destruction of erythrocytes, leucocytes and other cells under pathological conditions. If the cell activity is impaired or damaged, the cell membrane becomes permeable or it ruptures.

The cell contents including their enzyme components are released into intracellular fluid and eventually reach the plasma. If a large volume of cells are effected the plasma level of these non functional enzymes increase suddenly (Praful, 1994). If any organ is injured, the functional cells may spill the enzymes into blood, thereby increasing enzymatic levels in the blood and signaling the organ damage. Enzymes are proteins and found throughout the organs of the body, each with a
unique function. Among the functional enzymes that can be detected in diseased condition are aminotransferases. The name aminotransferases is derived as they catalyze chemical reaction in the cells in which an amino group is transferred from a donor molecule to a recipient molecule. They include AST and ALT. AST present in blood (serum) is known as serum glutamic oxalo acetic transaminase (SGOT) and ALT in blood (serum) as glutamic pyruvic transaminase (SGPT). AST (SGOT) is found in majority of tissues like liver, heart, muscle, kidney and brain. AST plays a major role in the metabolism of the amino acid, alanine. It is a mitochondrial enzyme which is predominantly found in liver, skeletal muscle and kidney.

It is released into serum during tissue damage. ALT (SGPT) is normally found in larger concentrations. ALT helps to neutralize the protein. It is a cytosolic enzyme and more specific to liver. It is released into the blood stream during cell injury. The highest levels of AST and ALT are found with diseased disorders and/or the death of numerous liver cells (intensive hepatic necrosis). Liver damage can be assessed by estimating the level of serum enzymes like AST, ALT and ALP which are originally present in higher concentration in cytoplasm. Hepatopathy leads to leakage of enzymes into blood stream relating to liver damage (Rajeswary et al., 2011).

Normally, the transaminase levels are found in low concentrations in blood during extensive tissue destruction; these enzymes are liberated into the serum. Liver tissue is rich in both transaminases, but it contains more of ALT than of AST although both transaminases are elevated in sera of patients with acute hepatic diseases, ALT, which is only slightly elevated in cardiac necrosis, is therefore a more specific indicator of liver damage. In both hepatic and post hepatic conditions high levels of ALT are observed. Phosphatases are a group of enzymes which are characterized by
their ability to hydrolyze different organic phosphates such as p-nitrophenyl phosphate, phenyl phosphate and sodium beta glycerol phosphate.

Clinically three types of phosphatases are recognized – alkaline phosphatase (ALP) (of serum, bone, liver, and intestine), acid phosphatase (ACP) (of prostrate, liver and serum) and cell phosphatase (CP). Lysosomal imbalance may result in the destruction of intact membranes (Abraham and Wilfred, 2000). ALP is a membrane bound enzyme related to the transport of various metabolites (Coleman, 1992). It is the marker enzyme for plasma membrane and is required in large amount for proper functioning of organ. ALP has been described as an enzyme in the dephosphorylation of lipopolysaccharides (LPS) (endotoxin) under physiological conditions both \textit{in vivo} and \textit{in vitro} as a natural response to detoxify and neutralize LPS (Poelstra \textit{et al.}, 1997). ALP is a wide specificity enzyme, which also catalyses transphosphorylation.

In humans and other mammals, atleast four distinct but related ALPs are known. They are intestinal, placental, placental-like, and liver/bone/kidney (or tissue non-specific) alkaline phosphatase. The first three are located together on chromosome 2 while the tissue nonspecific form is located on chromosome 1. The exact physiological functions of ALPs are not known, but appears to be involved with a large number of physiological processes, (the detoxification of LPS).The biologic function of ALP is uncertain but it has been suggested that ALP plays an important role in cellular transport (Russell \textit{et al.}, 1972), proliferation and differentiation (Karasaki, 1975) and in the regulation of cell metabolism and gene transcription (Huang \textit{et al.}, 1976).

ALP is present in several different organs, e.g., placenta, liver, intestine, and its metabolism can be inhibited under pathogenic conditions (Fishman and Ghosh, 1967; Moss, 1974). Focal loss of ALP has been reported in both mouse and rat
urinary bladder during treatment with chemical carcinogens (Highman et al., 1975; Kunze et al., 1975). A high level of ALP was found in human bladder tumor cell lines (Benham et al., 1977). The activity of ALP appears to be associated with structures which play a role in the absorption of fats or glucose. Activity of ALP in the golgi apparatus, in the cells of intestinal villi, in kidneys and in skeletal muscles of mice and rats has been investigated by cytochemical and biochemical methods (Clark, 1961; Mizutani and Barrentt, 1965; Hugon and Borgers, 1966).

In mammals, there are two forms of ALPs, one of which is distributed in intestine and the other is widely distributed in a variety of tissues (Van Belle, 1972; Goldstein et al., 1980). Ponder and Wilkinson (1981) examined various tissues such as thymus, spleen, lymph nodes, Peyer’s patches, tongue, salivary gland, oesophagus, stomach, small and large intestine, liver, pancreas, lung, kidney, thyroid, brain, nerve, muscle and skin for the endogenous ALP staining and found in most tissues; levamisole inhibits the non intestinal form of ALP without affecting the intestinal form. Significant activity of ALP has been reported in some cases of myeloid metaplasia, infection, leukocytosis, leukemoid reactions, trauma, surgery and various stressful conditions (Valentine et al., 1951). It is low in conditions such as pernicious anemia and the release of enzyme may be influenced by changes in the intracellular environment (Tanaka et al., 1960; Rosner and Lee, 1965).

Drugs which interfere with the synthesis of RNA or protein bring about an elevation of ALP activity in mice (Moog and Grey, 1967). The localization of ACP and ALP in the duodenum of the rat, the hamsters and the guinea pig was investigated and it was assumed that ALP follows a catabolic pathway and is finally degraded in lysosomes (Hugon and Borgers, 1968; Hoshi et al., 1997). Marked activity of ALP was found in mouse tumor cells (Bernstine et al., 1973). Intestinal inflammation
produces an induction of ALP activity and tissue non specific ALP is increased distinctly in enterocytes of mammalian cells by oxidative stress (via changes in glycosylation) (Sanchez de Medina et al., 2004; Lopez-Posadas et al., 2011). Increased activities of serum AST, ALT, ALP, total serum albumin and Malondialdehyde (MDA) was found in rats treated with a over dose of artesunate; hepatotoxicity and hemotoxicity caused by the drug are directly associated with the toxicological influence of drug administration on the biochemical integrity of the liver and RBC (erythrocytes is the primary sites of action) (Omotuyi et al., 2008).

Glutamate oxaloacetate transaminase and alkaline phosphatase activities increased in the serum of Trypanosoma brucei infected rats due to damage in liver and other organs where these enzymes may be abundant (Ekanem, 2005). Hyperlipemia and hepatic metabolism was studied in hyperlipemic albino rats maintained on a high fat diet (Alisi et al., 2008). Aqueous extract (100, 200, 300mg/kg/day) of Urtica dioica reduced dyslipidemia and restored hepatic chemistry in hyperlipemic animals. The extract was effective in normalizing the atherogenic lipoprotein phenotype. Total cholesterol (CHOL), Triglyceride (TG), Low density lipoprotein cholesterol (LDL), High density lipoprotein cholesterol (HDL), LDL/HDL-ratio, and Total Non-HDL cholesterol (TNH- CHOL) were significantly reduced by the treatment. There was no significant effect of treatment (with extract) on HDL. Hyperlipemia was associated with significant elevation in serum liver enzyme (ALT, AST, LDH and -GT) activities that are markers of altered hepatic chemistry. A cell surface glycoprotein (CD36) is found in many cell types including platelets, endothelial cells, and monocytes (Asch et al., 1987), differentiated adipocytes (Harmon and Abumrad, 1993), mammary epithelial cells (Greenwalt et al., 1990), and intestinal enterocytes (Drover et al., 2005; Nassir et al., 2007).
In mice (Dobozy et al., 1990), rats (Vega et al., 1991), humans (Tandon et al., 1989), and in other mammals (Berglund et al., 1996), CD36 binds a broad spectrum of extracellular ligands, including thrombospondin-1 (Asch et al., 1987), oxidized LDL (Endemann et al., 1993), collagen (Tandon et al., 1989), and Long chain fatty acids (LCFAs) (Baillie et al., 1996; Ibrahimi et al., 1996). Among numerous functions, CD36 has been demonstrated to play a role in facilitation of the transport of LCFAs into adipocytes (Harmon and Abumrad, 1993), platelets (Salah-Uddin et al., 2002), skeletal muscle cells (Bonen et al., 1998), cardiomyocytes (Bastie et al., 2004; Bonen et al., 2004; Hwang et al., 1998; Kintaka et al., 2002; Nozaki et al., 1999; Tanaka et al., 2001; Watanabe et al., 1998) and enterocytes (Nassir et al., 2007). The interactions between CD36 and intestinal alkaline phosphatase (IAP) may be important for efficient fat transport in mouse intestine (Lynes et al., 2011).

Mammalian ALPs comprise upto four distinct families, including IAPs (Narisawa et al., 2003; Millian, 2006; Lalles, 2010). IAPs have been implicated in the innate immune responses of the mammalian intestine and, in local pH homeostasis, along the brush border (Lalles, 2010).

IAP detoxifies a variety of bacterial toxins, including lipopolysaccharides (LPS), and flagellin (Chen et al., 2010). Furthermore, it has been reported that inhibition of endogenous IAP by L-phenylalanine (Phe) increases serum endotoxin levels (Koyama et al., 2002), based upon these observations regarding IAP function, it is said that ALP could play an important role in preventing gut-derived systemic inflammation. Endogenous IAP plays a critical role in reducing endotoxemia, and oral supplementation with IAP prevents high fat diet (HFD)-induced endotoxemia, as well as metabolic syndrome in mice (Kaliannan et al., 2013). Inhibition of endogenous ALP by levamisole significantly reduces survival of rats intraperitoneally injected
with *E. coli* bacteria, whereas this drug does not influence survival of rats receiving a sublethal dose of the gram-positive bacteria *Staphylococcus aureus*, illustrating a crucial role for this enzyme in host defense. The effects of levamisole during gram-negative bacterial infections and the localization of ALP as an ecto-enzyme in most organs as well as the induction of enzyme activity during inflammatory reactions and cholestasis is in accordance with the protective role.

Increased serum ALP levels are associated with hepatic damage. Upon an endotoxin insult, circulatory ALP is redirected to hepatocytes, thereby reducing circulatory ALP levels (Bentala et al., 2002). Hepatocytes also remove the LPS-loaded chylomicrons (Harris et al., 2002) rapidly from circulation with a half life of 5 - 10 minutes. LPS is next removed through biliary excretion, thereby preventing Kupffer cells, being a major target for circulating LPS to become activated. In patients suffering from septicaemia, it has been observed that increased serum ALP may be preceded by reduced ALP serum levels and that circulating ALP would be cleared from circulation upon LPS interaction (Bentala et al., 2002).

The increase in subsequent ALP- levels therefore, may be a feedback mechanism in response to this ALP reduction. ALP exerts its catalytic activity towards LPS primarily in the vicinity of a membrane, possibly in so-called lipid rafts. ALP and ACP activity has been reported to be associated with several different structural elements of the rat submandibular gland such as capillaries (Gomori, 1941; Bruce and Bogart, 1968), the acini and blood vessels (Arvy, 1963; Kronman, 1963), the basement membrane of serous elements (Burstone, 1961), and the myothelial cells (Leeson, 1956, 1957; Tamarin, 1966). ACP is frequently employed as a marker enzyme to assess the lysosomal changes *in vivo* because it is localized almost exclusively in the particle and its release parallels that of lysosomal hydrolyses.
The activity of ACP/ml. of blood in dystrophic mice was approximately seven times the activity/g.wet wt. of normal muscle. ACP, cathepsin and glucose 6-phosphate dehydrogenase showed increased activity in dystrophic muscle. Increase of enzymes could result from damage to muscle lysosomes, (with the release of the enzymes); such damage may be an important factor in the cause of muscular dystrophy. However, there is no good evidence that muscle fibers contain particles which serve as reservoirs of hydrolytic enzymes, corresponding to the lysosomes of liver. Moreover, homogenizing the tissue in water or treatment with detergent would, in any case, be expected to release the enzymes, if such bodies behaved like liver lysosomes.

Most of the researchers agree that phagocytosis is a feature of the breakdown of dystrophic muscle. Consequently the increase of enzyme may be due to enzymes in the macrophages. Rubenstein and Smith, (1962) showed histochemical evidence that macrophages in necrotic lesions of various tissues contain high content of these enzymes. ACP activity in the dystrophic muscle was, on the average, about 50% higher than normal one (Pennington, 1962). ACP and $\beta$-glucuronidase were significantly increased in the injured muscle of rabbits at 7 days after ischaemia.

At this time regenerative processes were evident in the muscle and particularly high levels of $\beta$-glucuronidase activity were visualized within muscle fibers. It is suggested that $\beta$-glucuronidase may have an anabolic role in the early regenerative processes of muscle. Some acid phosphatase activity was also visualized within muscle fibers at 7 days. The biochemical activities of both enzymes approached normal values in the injured muscle at 14 days after ischaemia but residual activities were still evident histochemically at 28 days (Shannon et al., 1974). In the cholesterol-fed rabbit, the change from normal muscle cell to foam cell is
accompanied by marked physical and chemical changes of the lysosomes, including their progressive over-loading with cholesteryl ester. Small diaminobenzidine-positive particles were present in normal smooth muscle cells and in those at all stages of foamy transformation. These particles were more frequent in foam cells, in agreement with the marked increase in catalase activity in these cells (Shio et al., 1974).

ACP reaction product was restricted to small vesicles located in the cytoplasm or near the golgi apparatus and in aortic smooth muscle cells in normal rats (Wolinsky et al., 1973). ACP activity has been demonstrated in rat liver with the semipermeable membrane technique using naphthol, AS-BI phosphate as substrate and hexazoitized pararosaniline (HPRA) as simultaneous coupling agent. ACP activity is 1.2 times higher periportally than pericentrally in rat liver, and that 24 hr fasting before the experiments did not change the ACP activity (Frederiks et al., 1987). Lysosomal acid phosphatase (LAP) has a unique function in only a subset of cells, where its deficiency causes the storage of a heterogeneously appearing material in lysosomes (Saftig et al., 1997). Administration of lead caused alteration in the activity of phosphatases and the level of creatinine in heart, liver and skeletal muscles of mice due to cellular destruction and encapsulation. The activity of phosphatases and the level of creatinine in heart, liver and skeletal muscles altered significantly in lead treated mice (Satyalatha and Vardhani, 2000). Mice exposed to hookworm infection showed disturbances in liver ALP and ACP (Satyalatha and Vardhani, 2005).

Prostatic acid phosphatase (PAP) suppresses pain by functioning as an ecto-5’-nucleotidase. The molecular and physiological functions for PAP in purine nucleotide metabolism suggest PAP in the treatment of chronic pain (Zylka et al., 2008). Pyridoxal-5-phosphate (PLP), the active form of vitamin B₆ catalyzes a large array of reactions in the synthesis, catabolism, and interconversion of aminoacids (Snell, 1990;
Leklem, 1991; Denesyuk, 2002). The cellular content of PLP is determined by the function of pyridoxal kinase (PK), PLP oxidase and phosphatases; both kinase and oxidase play a central role in vitamin B₆ metabolism (McCormick and Chen, 1999). PK has been detected in virtually all mammalian tissues and a number of enzymes including AST, ALT, Glutamate decarboxylase (GAD) depend on PLP for their activities (DeRosa and Swick, 1975; Kim et al., 1988; Cheung et al., 2003).

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The synthesis of vitamin B₆ dependent apoenzymes in the presence of phosphatase is of interest. The synthesis of AST was inhibited during strain (Mizuguchi et al., 2001; Islam et al., 2003). AST is a mitochondrial enzyme present in the liver parenchymal cells and may also be released from heart, liver, skeletal muscle and kidney (Kumar et al., 2008). Female mice infected with single doses of *Ancylostoma caninum* larvae showed a marked increase of AST and ALT (Vardhani, 1986). The biochemical and molecular hepatotoxicity induced by aluminium chloride (AlCl₃) and the protective role of saffron and honey against such toxicity resulted in a significant increase in the cholesterol levels, triglycerides, GGT, ALT, AST, ALP, lipid peroxidation, and presence of hyperglycemia in the AlCl₃ group of mice when compared to the control. However, treating those mice exposed to AlCl₃ by saffron and honey improved the disrupted liver biochemical markers and alleviated the increase of lipid peroxidation. ALT, AST and ALP are considered indicators of hepatocellular health (Vozarova et al., 2002; Yang and Chen, 2003).

Increase of AST and ALT, superoxide dismutase, catalase and glutathione peroxidase was found in liver and kidney of ethanol treated rats (Saravanan et al., 2002). A significant rise in ALP, AST and ALT activities was found in the atherogenic diet group of rats (Naik and Sheth, 1978; Deepa and Varalakshmi, 2004). Stronger Neo- Minophagen C (SNMC) has been shown to significantly lower AST,
ALT and GGT concentrations with simultaneously ameliorating histologic evidence of necrosis and inflammatory lesions in the liver (Steve, 2005; Van Rossum et al., 2001). Transaminases and phosphatases are critical enzymes in biological processes; they perform detoxification, metabolism and biosynthesis of energetic molecules for different essential functions. Dysfunction of these enzymes causes biochemical impairment and lesions of tissue and cellular function. The increased enzymes observed in acrylamide-treated mice reflect cellular damage induced by toxin (Rawi, 1995; Yousef and El-Demerdash, 2006). *Withania somnifera* extract protected liver cells against oxidative stress induced by lead intoxicification (Chaurasia et al., 2000) and restored the normal level of AST and ALT in dimethoate (DM) treated guinea pigs (Ju et al., 2008). Significant decrease of serum proteins and increase of transaminases was found in broilers treated with aflotoxin B1 (Madhuri et al., 2009). Administration of *Aframomum sceptrum* tends to normalize ALT, AST and ALP levels (George et al., 2010). The use of saffron and honey minimized the toxic effect on the biochemical and molecular levels (Shati and Alamri, 2010).

Activity of AST and ALT increased in the serum of acrylamide-treated rats (Sabik, 2011). The hepatoprotective activity of aqueous extract of *Lawsonia intermis* was evaluated against paracetamol induced liver damage in rats (Selvanayaki and Ananthi, 2012). The plant aqueous extract was effective in protecting the liver against the injury induced by paracetamol in rats; this was evident from significant reduction in serum enzymes like ALT, AST, ALP, ACP and protein and bilirubin. Gamma irradiation caused a marked increase in serum AST and ALT levels indicating liver injury and these changes were ameliorated by using *W. somnifera* extract (Mansour and Hafez, 2012). Administration of Clarithromycin (Clarinic) induced changes in the activities of ALP, ALT, AST and gamma glutamyl transferase (GGT) in the plasma of
rats treated with two doses of the drug (Olayinka and Ore, 2012). D-galactosamine/lipopolysaccharide (D-GalN/LPS) intoxicated rats showed a significant increase in the activities of serum marker enzymes such as AST, ALT and ALP while there was a significant inhibition of DNA, RNA and protein contents in rat liver tissues (Fyiad et al., 2012). Oils from Zinger officinale and Curcuma longa (200mg/kg) exhibited hepatoprotective activity by decreasing the activities of serum AST, ALT and ALP in acute ethanol-induced fatty liver in male albino rats (Nwozo et al., 2014).

Vitamin E is the most effective lipid soluble antioxidant found in the biological system which prevents the initiation of oxidative tissue damage. Administration of vitamin E significantly reduced the elevated levels of serum AST and ALT thereby suppressing the liver injury induced by acrylamide fed mice (Siahkoohi et al., 2014). Investigations on the hepatoprotective effects of Triphala in D-Galactosamine (D-GalN) induced hepatic toxicity in mice explain that D-GalN induced hepatic damage resulted in a significant increase in the activity of ALT, AST, ALP, bilirubin, lipid peroxidation (LPO) and Tumour necrosis factor (TNF-α) level with a decrease in the levels of anti-oxidant enzymes such as SOD, CAT, glutathione peroxidise (GPx), glutathione reductase (GR), glutathione-s-transferase (GST) and total reduced glutathione (Sabina et al., 2013).

Hepatotoxicity was induced by thioacetamide (TAA) of 100mg/kg.s.c. and Lannea coromandelica bark extract (LCBE) at different doses of 400 and 200 mg/kg were orally administrated to male wistar rats. TAA caused elevation of serum AST, ALT, ALP when compared to normal (Rao et al., 2014). Role of W. somnifera extract in attenuation of dimethoate (DM) caused a significant hepatic protection against DM-induced oxidative damage in male guinea pigs as observed from the elevation of hepatospecific enzyme activities (A1- Awthan et al., 2014). Increased level of liver
AST and ALT was found in mice treated with IDS + vaccine compared to IDS treated and control animals (Sridevi, 2011).

Glutathione plays an important role in the regulation of protein synthesis as well as protein degradation. Particularly in the liver, glutathione is also involved in detoxification and metabolism of a number of substances. In addition, glutathione is also involved in transmembrane transport of amino acids, in particular cysteine, which has traditionally been regarded as the limiting amino acid for glutathione metabolism as well as for protein synthesis. (Meister and Andersson, 1983; Deneke et al., 1989; Dolphin et al., 1989; Taniguchi et al., 1989). The importance of glutathione is mainly related to the clinical picture in individuals suffering from enzymatic deficiencies in the biosynthesis of glutathione (Larsson, 1989). The liver seems to be the central organ in glutathione metabolism, and plasma and erythrocyte concentrations are thought to reflect the synthetic capacity of the liver. In other organs, the origin, biosynthesis and turnover of glutathione is more obscure (Adams et al., 1983; Lauterburg et al., 1984; DeLeve and Kaplowitz, 1990). In addition to the synthesis and degradation of glutathione, this molecule undergoes changes between the reduced and oxidised state. Oxidised glutathione (GSSG), formation leads to depletion of the intracellular pool of glutathione. Another pathway of glutathione depletion is via the reaction of GSSG with cellular proteins which are then degraded. In muscle, free glutamine pays a central role in amino acid metabolism. It is produced at a high rate and exported from muscle mainly to organs or cells. Free glutamine is maintained at a very high concentration in skeletal muscle and a significant correlation between glutamine concentration and muscle protein synthesis has been reported in malnourished and endotoxaemic rats (Jepson et al., 1988).
In human subjects, a significant correlation between the change in glutamine and the change in protein synthesis was found following surgical trauma (Wernermann et al., 1990). Furthermore, a correlation between glutamine and protein degradation has also been reported in an experimental rat muscle system (MacLennan et al., 1988). Although a significant relationship between protein metabolism and glutamine concentration in muscle has not been established, at least in some situations the interaction between glutamine and protein metabolism can be regulated possibly via glutathione. GST-fused proteins could exhibit biological activity in living cells because as shown in numbers of microinjection experiments in which protein functions have been analysed by using GST-fused proteins (Sasaoka et al., 1996a, b; Klockow et al., 2000; Namiki et al., 2003; Murata et al., 2008).

In the detoxification of these reactive free radicals, some of the GSH-related enzymes such as glutathione-s-transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GPx) are involved in the intracellular defense mechanisms (Tew, 1994; Ohkuwa et al., 1997; Noctor and Foyer, 1998; Teramoto et al., 1999). GSTs are the major phase II detoxification enzymes mainly found in the cytosol. In addition to their role in catalyzing the conjugation of electrophilic substrates to glutathione (GSH), these enzymes also carry out a range of other functions. To make compounds more hydrophilic, it conjugates GSH to electrophilic compounds and improves their excretability (Hayes et al., 2005). Majority of GST substrates are either xenobiotics or products of oxidative stress that are toxic to cells and often carcinogenic. The cytosolic GST isozyme of rodents and humans can be grouped in several classes, such as α, μ, π, θ, ω and ζ based on their aminoacid sequences, immunological properties and substrate specificities (Townsend and Tew, 2003).
They have peroxidase and isomerase activities and are involved in protection of cells against H$_2$O$_2$-induced cell death (Sheehan et al., 2001).

GST is a ubiquitous enzyme, which provides cellular protection against a wide variety of xenobiotics. GST-µ1, *GSTM1*, belongs to a superfamily of glutathione S-transferase that metabolizes a broad range of reactive oxygen species (ROS) and xenobiotics. There are 8 distinct classes of soluble GSTs that have been identified according the substrate specificity, chemical affinity, structure, and kinetic behavior of the enzyme (Landi et al., 1998; Strange et al., 1984). In diseased states such as atherosclerosis and arterial injury-induced neointimal hyperplasia, it is thought that a key component involves medial smooth muscle cell proliferation and migration into the arterial intima (Yang et al., 2009). GSTs are part of phase II metabolism of xenobiotics, a process which removes toxic compounds. Oxygen is required to maintain life and metabolic processes including drug metabolism but certain destructive oxygen derivatives named ROS are generated during oxygen use (Gupta et al., 2007). Certain antioxidants protect the body against the damages caused by ROS and maintain redox homeostasis; these include both enzymatic SOD, glutathione peroxidase, catalase, etc) and non-enzymatic (glutathione and vitamins A, E and C) antioxidants. Oxidative stress sets in when the redox balance is disrupted by extreme generation of ROS or when the antioxidant capacity is insufficient (Thomas, 2000; Golden et al., 2002). Numerous studies have shown that muscle cells also release superoxide into extracellular space (Reid et al., 1992; McArdle et al., 2005).

Oxidative stress has been implicated in lipid peroxidation, protein and DNA damage and in the pathogenesis of certain diseases (Aruoma, 1999; Berlett and Stadtman, 1997; Wallace, 2002). Metabolism of some macrolide antibiotics may lead to generation of ROS or free radical intermediates and can cause depletion of
antioxidant reserve (Yazar et al., 2004, 2010; Er et al., 2011). Reactive oxygen species (ROS) such as hydroxyl radicals (OH), super-oxide anion radicals and hydrogen peroxide are extremely reactive and react with the molecule of cell membranes that are composed of a double layer of lipids with proteins dispersed throughout (Sadani and Nadkarni, 1997; Ho et al., 1998). Under normal conditions, there is a balance between the generation of ROS and the cellular antioxidant systems (Timoth and Sharma, 1991). Exposure to stress (due to infection/toxin) produces significant alterations in the oxidant activity in tissues and causes over production of ROS leading to oxidative damage of the lipids, proteins and DNA.

The oxidation of polyunsaturated fatty acids in membrane induced by ROS is called lipid peroxidation (LPO). ROS has been found to be involved in the toxicity of various organophosphorous pesticides (Bagchi et al., 1995; Mansour and Mossa, 2010). ROS-mediate damage could produce alterations of cellular macromolecules such as membrane lipids, DNA and proteins (Kehrer et al., 1990; Islam et al., 2012). However, organisms have protective systems against ROS, like endogenous antioxidant enzymes. Superoxide dismutase (SOD), glutathione peroxidase (GSh-Px) and catalase (CAT) constitute primary enzymatic defense system (Halliwell and Gutteridge, 1990). SOD is responsible for direct damage of biological macromolecules and for generating other reactive oxygen species. CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase and glutathione peroxidase were assayed in mouse islets and other tissues. Pancreatic islets were found to belong to tissues with relatively little activity of the protective enzymes (Grankvist et al., 1981).

Toxic products of oxygen reduction can also arise in the course of inflammation (e.g., Babior et al., 1973; Johnston et al., 1976; DeChatelet et al., 1977; Petrone et al., 1980; Sacks et al., 1978; McCord and Wong, 1979). SOD and CAT
had been shown to protect β-cells against the toxic action of alloxan (Grankvist et al., 1979; Fischer and Hamburger, 1980). If pancreatic islets were found to contain markedly lower endogenous activities of these enzymes as compared with other tissues, this relative lack of protection against oxygen-reduction products might explain why the β-cells are exceptionally vulnerable to alloxan.

SOD catalyses the disproportionation of $O_2^-$ that presumably arises in the autoxidation of dialuric acid: $2O_2 + 2H^+ \rightarrow O_2 + H_2O_2$ (Cohen and Heikkila, 1974). SOD is widely distributed in the cells with high oxidative metabolism and has been proposed to protect such cells against the deleterious effects of superoxide anion; it also keeps the concentration of superoxide radicals at low levels and therefore plays an important role in defense against oxidative stress (Fridovich, 1995). SOD and CAT are the two scavenging enzymes that remove toxic free radicals (Wohaieb and Godin, 1987). SOD is one of the most important enzymes that diminish the toxic effects of free radicals (Arunabh et al., 1999). Elevation of metabolism by exercise, results in a greater production of superoxide radicals (McArdle et al., 2005), which are dismutated to hydrogen peroxide by SOD. Numerous studies have shown that muscle cells also release superoxide into the extracellular space (Ried et al., 1992; McArdle et al., 2005). Free radical overproduction due to exercise occurs mainly in the active skeletal muscles (Vina et al., 2000; McArdle et al., 2001; Jackson, 2005). Catalase has been regarded as a major determinant of hepatic and cardiac antioxidant status (Wohaieb and Godin, 1987). It is involved in the detoxification of $H_2O_2$ concentrations (Yoshikawa et al., 1993, Manonmani et al., 2002). CAT is a tetrameric heme-containing enzyme complex (Forsberg et al., 2001; Rojkind et al., 2002).

Catalases are essential components of the cellular equipment to cope with oxidative stress (Chagas et al., 2009). Among several potentially harmful reactive...
oxygen species (ROS), hydrogen peroxide is probably the most abundant, occurring in the cells and in their environment. H$_2$O$_2$ is produced intracellularly as a by-product in reactions catalysed by several oxidases (Scandlios, 2002). Catalases are enzymes capable of consuming H$_2$O$_2$ reducing the harmful effect of this reactive oxygen compound and protecting the cellular environment against oxidative stress. The catalytic reaction by catalases takes place in two steps; first the H$_2$O$_2$ molecule oxidizes haeme to an oxyferryl species in which one oxidation equivalent is removed from the iron and one from the porphyrin ring to generate a porphyrin cation radical. Next, H$_2$O$_2$ is then used as a reductant of compound I to regenerate the resting state enzyme, water and oxygen. Catalases can also assume an inactive state, called compound II, a product of compound I, during exposure to its own substrate, H$_2$O$_2$ (Chance and Herbert, 1950). Terblanche, (1999) reported that CAT activity was higher compared to the rest in several tissues: liver, heart, kidney, or lung in male and in female rats. The role of oxidative stress in the mechanism of isoniazid and rifampicin-induced hepatitis has been reported by Attri et al., (2000).

SOD, CAT, and GST play an important role in the biological systems to act against oxidative stress (Akyol et al., 2002). CAT and SOD activities in rat lung tissue was increased significantly after the effort in young rats, a response that was significantly depressed in old rats suggesting a decrease in the antioxidant defense system (Hatao et al., 2006). The more efficient clearance of ROS however, requires the coordinate actions of antioxidant enzymes, such as SOD, and CAT (Rojkind et al., 2002). The heightened paraquat sensitivity in transgenic mice was associated with a profound decrease in the activities of antioxidative SODs and CAT (Gong et al., 2006). An increase in ROS-elicited oxidative damage to DNA and other biomolecules may impair normal functions of tissue cells and lead to human aging and disease
The bioenergetic function of mitochondria is decreased with age in the postmitotic cells (brain, heart and muscle) of the human and animals (Wei, 1998; Lenaz et al., 2000). Mn-SOD activity was increased with age but GPx and CAT activities did not show significant changes in human skeletal muscle (Wei et al., 2001). Mice treated with NaF for 14 days revealed decreased SOD, CAT and GST activities and increased xanthine oxidase (XOD) in brain and gastrocnemius muscle (Aiguo et al., 2004). A significant increase in the activities of plasma insulin, SOD, CAT, GPx, GST and GSH was observed in the brain of rat on treated with Helicteres isora bark extract (HIBE) and tolbutamide (Kumar and Murugesan, 2007).

Reduction in the activities of antioxidant enzymes (SOD, CAT, GST and GSH) was observed in lung cancer bearing mice and these changes were reversed to near normal with Quercetin supplementation (Kamaraj et al., 2007). SOD activity was decreased in both liver and kidney whereas CAT was increased only in liver but GST increased in both liver and kidney in cadmium exposed rats and reversed on selenium administration (Ognjanovic et al., 2008). The decreased activities of key antioxidant enzymes such as SOD, CAT, GST and GSH in diabetic rats were brought to normal upon HI (Helicteres isora) treatment (Kumar et al., 2008). Hao et al., (2009) reported that purslane can be used as a medicinal plant where it is used for anti-aging, thereby increasing the level of SOD and decreasing the level of MDA in the brains of mice treated with D-galactosamine. The antioxidant enzymes such as SOD and GST take part in maintaining GSH homeostasis in tissues (Abdel-Moneim et al., 2011).

The hepatic antioxidant enzyme activities were decreased in the liver of rats administered with CCl₄, activities of SOD, CAT, and GST were restored by Decalepis hamiltonii (DHA treatment) (Srivastava and Shivanandappa, 2010). Catalase and SOD activities increased significantly in liver, kidney and testis of purslane.
*Portulaca oleracea* treated albino rats (Dkhil *et al*., 2011). Significant depletion in glutathione content (GSH), superoxide dismutase (SOD) and catalase (CAT) activities in the serum of rats exposed to two different doses of gamma radiation that produced oxidative stress (Saad and Ammar, 2011). Decreased activities of hepatic antioxidants like SOD and CAT were observed in D-galactosamine/ lipopolysaccharide (D-GalN/ LPS) intoxicated rats (Fyiad *et al*., 2012). 50% ethanolic extract of *Cissampelos pareira* (CPE) on CAT, SOD, and lipid peroxidation against control and rifampicin (RIF) + isoniazid (INH) induced hepatotoxicity in rats (Verma and Hussain, 2013).

A significant decrease in myocardial GSH level along with decrease in the activities of glutathione dependent enzymes (GPX, GST, GPx), antilipid peroxidative enzymes (SOD and CAT) and increase in lipid peroxidation products was found in heart tissue of isoproterenol (ISO) administered rats. Pretreatment with *Tribulus terrestris* fruit aqueous extract (TTFAEt) prevented these adverse changes (Sailaja *et al*., 2013). The activity of GST was significantly decreased in the liver and colon mucosa of 1, 2-dimethylhydrazine (DMH) alone treated rats, while linalool supplementation enhanced the GST activity and increased the activities of SOD and CAT thereby enhancing the detoxification of carcinogens (Srithar *et al*., 2013). Cyclophosphamide (CP)-induced oxidative stress in the liver by reducing the activities of SOD, CAT and GST which were mitigated with *Decalepis hamiltonii* (DHA) treatment (Zarei and Shivanandappa, 2013). Oils from *Zinger officinale* and *Curcuma longa* and at a dose of 200mg/kg showed hepatoprotection by decreasing the activities of serum enzymes and significantly restoring the activities of GST and SOD (Nwozo *et al*., 2014). Preventive effects of citrus flavanoid hesperidin (HDN) against acrylamide induced neurotoxicity in male wistar rats by increasing the activity of SOD and CAT in the brain tissue (Kumar *et al*., 2014).

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Decreased CAT and SOD activities were observed but highest CAT activity was observed in kidney when compared to brain and liver whereas highest SOD activity was observed in liver when compared to kidney and brain and increased GST activity was observed but highest elevation was seen in brain when compared to kidney and liver in sodium fluoride (NaF) exposed mice (Sandeep et al., 2014).

From the literature available it is known that viral hepatitis may cause abnormalities in the body’s metabolism and immune response and/or cellular damage in vital organs. Very little information is known with regard to biochemical, qualitative protein profile and histological changes in abdominal muscles due to viral infections. Therefore, the present studies were undertaken to analyse the effect of Gene Vac B vaccine in Immunex DS treated mice with regard to certain biochemical, and protein quality and histopathological changes in the abdominal muscles. The present work is designed to determine the following

1) Enzymatic activity of AST, ALT, ALP, ACP, GST, SOD and CAT, SDS PAGE analysis of protein and histopathology of abdominal muscles in mice treated with a single dose of Immunex DS (150mg/mouse) for one day and vaccinated with a single dose of 0.07 ml of Gene Vac B vaccine (on day 7).

2) Enzymatic activity of AST, ALT, ALP, ACP, GST, SOD and CAT, SDS PAGE analysis of protein and histopathology of abdominal muscles in mice treated with a single dose of Immunex DS (150mg/mouse) for one day and vaccinated with a single dose of 0.1 ml of Gene Vac B vaccine (on day 7).

3) Enzymatic activity of AST, ALT, ALP, ACP, GST, SOD and CAT, SDS PAGE analysis of protein and histopathology of abdominal muscles in mice treated with a single dose of Immunex DS (150mg/mouse) for one day and vaccinated with a single dose of 0.2 ml of Gene Vac B vaccine (on day 7).
4) Enzymatic activity of AST, ALT, ALP, ACP, GST, SOD and CAT, SDS PAGE analysis of protein and histopathology of abdominal muscles in mice treated with a single dose of Immunex DS (150mg/mouse) for one day and vaccinated with a single dose of 0.4 ml of Gene Vac B vaccine (on day 7).

5) Enzymatic activity of AST, ALT, ALP, ACP, GST, SOD and CAT, SDS PAGE analysis of protein and histopathology of abdominal muscles in mice treated with a single dose of Immunex DS (150mg/mouse) for one day and vaccinated with a single dose of 0.8 ml of Gene Vac B vaccine (on day 7).

6) Enzymatic activity of AST, ALT, ALP, ACP, GST, SOD and CAT, SDS PAGE analysis of protein and histopathology of abdominal muscles in mice treated with a single dose of Immunex DS (150mg/mouse) for one day and vaccinated with a single dose of 1.0 ml of Gene Vac B vaccine (on day 7).

7) Enzymatic activity of AST, ALT, ALP, ACP, GST, SOD and CAT, SDS PAGE analysis of protein and histopathology of abdominal muscles in mice treated with a single dose of Immunex DS (150mg/mouse) for one day and unvaccinated with Gene Vac B vaccine.

8) Enzymatic activity of AST, ALT, ALP, ACP, GST, SOD and CAT, SDS PAGE analysis of protein and histopathology of abdominal muscles in control mice (untreated with IDS + Gene Vac B vaccine).