Hepatitis B (viral infection) causes inflammation in the liver; HBV is attracted by the liver cells causing either acute or chronic hepatitis. Approximately 60% of people infected with hepatitis B do not develop illness as the body’s defense system is able to fight with the virus; thus people cannot attack by hepatitis B again. The chronic form of hepatitis B is more dangerous and can lead to complications like liver cirrhosis, failure and cancer. The HBV spreads from person to person through blood or body fluids. People suffering from acute hepatitis B show mild illness and the severity of symptoms experienced differ between individuals of different age and general health. The clinical symptoms include: fatigue, fever, headache, weight loss, decreased appetite, nausea and vomiting, dark brown urine and pain in the right side of abdomen (just below the ribs). Chronic hepatitis B can be treated with antiviral medication and/or prevented by immunization. Since 1985 the hepatitis B vaccine has been part of the National immunization schedule.

Immunity is the capability of the body to resist the entry of harmful microorganisms or viruses. Immunity involves both specific and non specific components. Innate or non specific immunity is the natural resistance with which a person is born. The adaptive immunity is characterized by the cells involved in humoral immunity (mediated by antibodies) and cell mediated immunity (involves T lymphocytes alone). One way to stimulate the immune system in the production of antimicrobial molecules is the application of immunostimulants that increase defense to bacterial and viral infections (by stimulating non specific immunomechanisms). The use of immunostimulants as dietary supplements can improve the natural defense of animals providing resistance to pathogens during stress conditions. In India, IDS is
widely used in shrimp culture (as a dietary supplement) and considering its benefit in boosting the animals innate defense system, the present investigations were designed to assess the effect of IDS, IDS + vaccination in male Swiss albino mice with regard to quantitative changes in the level of AST, ALT, ALP, ACP, GST, SOD and CAT, then qualitative assessment of protein profile and histopathological changes in abdominal muscles.

AST levels showed considerable increase during the entire experimental period in all the experimental groups (A to F) of mice (except from day 3-5 in group F and a same value on day 1 in mice of group A). ALT level also showed considerable increase during day 1 to 5 of experimental period in all the experimental groups of mice (except on day 5 in group A and from day 3-5 in group B) when compared with controls. In comparison with IDS treated mice, the level of AST (except on day 1 and 2 in groups A, B and D, and on day 1 in groups C and E and on day 3 to 5 in group F) and ALT showed increase in all the experimental groups of mice (except from day 1-5 in groups A and B, on day 1 in group C and same value on day 2 in groups C, E and on day 1 in group D).

The present investigations indicate that though the mice of groups A to F were pretreated with IDS, HBsAg might have brought intolerance of some inherited factors like glycogenolytic defect in the muscles (resulting in the significant increase of AST and ALT in vaccinated animals). It is stated that muscle fibers change their properties during new activities and/or abnormal physiological conditions (Pette and Vrbova, 1985). These results are comparable to that of Kugelberg, (1976) who also reported that the adaptive mechanism which exists in young rats may alter the transamination process in experimental mice treated with immunostimulant and/or vaccine. The present observations coincide with that Vardhani, (1986) and Madhuri et al., (2009)
who reported significant increase of serum transaminases in mice and in broilers during ancylostomiasis and aflatoxicosis. The present findings clearly suggest that the excessive production of free radicals and lipid peroxide might have caused the leakage of AST and ALT in muscles as suggested by Cromheecke et al., (2000). Also, the vaccine and/or the immunostimulant might have induced oxidative stress in muscles indicating the increase of AST and ALT level. Sridevi, (2013) revealed increase of AST, ALT, ACP and ALP and histopathological changes in liver of mice treated with HB vaccine and/or immunostimulant as indicators of liver damage. The present results indicate that HB virus might have sensitized the experimental mouse immune system leading to the impairment in the transamination.

Considerable increase in ALP levels during the entire experimental period in all the experimental groups of mice (when compared with controls) indicates the influence of gene vac B antigen on the abdominal muscles (though they were pretreated with IDS). The level of ACP showed considerable increase in groups A to F from day 1 to 5 of experimental period. Interestingly in mice received immunostimulant, the increased level of ALP and ACP almost remained constant from day 1 to 5 of experiment. Though there was no difference in the activity of ALP and ACP on day 1 in the experimental (groups A and B), there was a gradual increase of ALP and ACP from day 1 - 5 of experiment in groups B and C (except the ALP value on day 2 in group A). Peak values of ALP and ACP on day 5 of experiment in groups B and C and increased activity of ALP from day 1 to 5 in groups C, D, E and F also confirm the antigen (viral) and antibody reactions in the abdominal muscles of experimental mice. A constant level of ALP was maintained on day 1 and 2 in case of group F and ACP activity increased from day 1 to 5 in groups E and F (except the constant values in group D on day 1 and 2); this significant alteration (increase or
decrease) in the metabolism of enzymes in the abdominal muscles clearly indicate the host’s defense/immune mechanism against viral hepatitis. Increase or decrease of enzymes may be influenced by the stress caused by immunostimulant (group I) and/or due to the IDS + vaccine in case of groups A, B, C, D, E and F. Abnormality in the synthesis of phosphatases might have caused by the glycogenolytic defect in the muscle of all the experimental mice. Whether, and to what extent, these factors are responsible for disturbed enzymatic metabolism in muscle is unknown.

Griggs, (1964) suggested that lead poisoning may inhibit the enzyme δ-aminolevulinic acid dehydrate (the most sensitive enzyme in the pathway) during the metabolism of phosphates. Satyalatha and Vardhani, (2005) also suggested the direct effect of lead in the synthesis of phosphatases in liver, heart and skeletal muscles of mice. The high ACP activity in the abdominal muscles of experimental mice also confirm that of Sleyster and Knook, (1982) who correlated the high ACP activity with higher lysosomal activity of kupffer cells in rats and Stoward et al., (1982) in skeletal muscles of rats. These observations compare well with that of Irintchev and Wering, (1987) who explained a possible adverse physiological mechanism in necrotic muscle fiber’s and/or in stressful conditions. Also, the existence of such adaptive mechanism is reported in young rats (Kugelberg, 1976). It is interesting to note that adaptive mechanism is playing a central role in the alteration of phosphatase levels in experimental mice treated with immunostimulant and/or vaccine.

The present investigations clearly indicate that the metabolic/pathogenic changes that occur in host immunity due to vaccine might have caused impairment in the synthesis of phosphatases. These results confirm that of Satyalatha and Vardhani, (2004) who related significant increase of phosphatases in skeletal muscles of immunosuppressed mice (treated with lead and also, in those treated with nematode
infective larvae and lead) due to lead poisoning. Fyiad et al., (2012) evaluated the protective effect of pomegranate juice against oxidative stress in rats during hepatitis infection. GST showed a considerable increase in all the experimental groups of mice (except from day 3 to 5 in group D and a same value on day 1 in A and C) when compared with controls. Interestingly in mice received immunostimulant, the level of GST increased from day 1 to 5 of experiment (except from day 1 to 3 in group A, on day 1 and 2 in group B, on day 1 in C, E and the entire group D). Peak values of GST were found on day 5 of experiment in groups A, C, E and F.

SOD showed a considerable increase in all the experimental groups of mice when compared with controls (except the same value of SOD on day 2 in A) and IDS treated mice (except same value on day 1, 3 in group A and day 1 and 2 in group B on day 1 in group C). Whereas, decreased CAT activity was found in all the groups of mice during the entire experimental period (except from day 3 to 5 in groups A and C, on day 4 and 5 in group D and on day 5 in group F) when compared with controls (group U) and immunostimulated mice (except on day 4 and 5 in groups A and D, from day 3 to 5 in group C, and on day 5 in group D).

Altered synthesis of transaminases, phosphatases, GST, SOD and CAT in various experimental groups of mice would suggest the marked inflammation, tissue damage and leakage of enzymes. These results compare well with that of Sakunthala et al., (2014a) who reported marked changes in the protein and DNA profile of stomach in IDS + vaccine treated mice. The marked alterations of enzymes/qualitative protein profile in the abdominal muscles of mice treated with IDS + vaccine in comparison with controls suggest that the administrated vaccine disturbed the synthesis of enzymes/proteins. Animals (group I) treated with immunostimulant (IDS) showed significant increase (statistically significant) (with
few exceptions) in the levels of AST, ALT, ALP, ACP, GST, SOD and CAT in comparison with controls (group U). These results suggest that the IDS slightly altered the synthesis of transaminases, phosphatases, GST, SOD and CAT in the abdominal muscles of mice. In all the IDS + vaccine treated experimental groups (A, B, C, D, E and F) of animals, the level of transaminases, phosphatases, GST, SOD and CAT is higher (with few exceptions) than IDS treated animals. This indicates that the host immune system is subjected to stress, thereby showing abnormality in transamination, dephosphorylation and detoxification in muscle cells. Although the mechanism of IDS with regard to the synthesis of enzymes is poorly understood, it is clear that the HBsAg antigen might have caused disturbance in the synthesis of AST, ALT, ALP, ACP, GST, SOD and CAT in the abdominal muscles of all experimental mice which received IDS + vaccine (though they were pretreated with IDS).

The increase of transaminases and phosphatases in experimental mice (IDS treated + vaccinated) suggests that the various single doses of vaccine might have caused adverse effects on the synthesis and storage of enzymes in the abdominal muscles. These results also explain that pretreatment with IDS is able to induce protection in muscle cells in the synthesis of AST, ALT, ALP, ACP, GST, SOD and CAT (though the experimental mice were injected with varied doses of Gene Vac B vaccine). Although, there was an increase in the level of transaminases and phosphatases in IDS treated + vaccinated group of mice, this rise is comparatively higher than controls. It is of interest to note that the increase of transaminases and phosphatases, and antioxidant enzymes was not on the dose (vaccine) dependent manner in all the experimental groups of mice (IDS treated + vaccinated). Though liver is the main site of biotransformation and it reduces the toxicity of toxins (Hodgson, 2004), the toxins released in the host system damaged the muscle cells.
causing pathogenicity. ALT (cytosolic enzyme) which helps to metabolize protein is more specific for the liver than AST.

An increase of AST level also may indicate cellular damage. Increased level of AST, ALT, ALP, ACP, GST, SOD and CAT (with few exceptions) in all the experimental groups of mice confirm the findings of Poli et al., (1987) and Paliwal et al., (2009) who found increased level of liver transaminases in rats during toxicosis. In the present investigations the toxicity induced by viral antigens may appear to involve generation of ROS or alteration in the level of GST, SOD and CAT. The increased level of GST and SOD and decreased level of CAT in experimental groups of mice suggests that these enzymes are synthesized to remove harmful ROS and impairment of abdominal muscles. Yazar et al., (2004, 2010) and Er et al., (2011) suggested that toxins may lead to the generation of ROS or cause depletion of antioxidant reserve.

When the antioxidant capacity is insufficient it may also lead to the abnormal synthesis of antioxidant enzymes/free radicals. Oxidative stress has been implicated in DNA damage and in the pathogenesis of certain diseases Wallace, (2002). The elevated level of SOD and decreased level of CAT in the experimental groups of mice may be due to the leakage of enzymes in muscle fibers and loss of functional integrity of membranes in abdominal muscles. The role of hepatic antioxidant defense system has been reported by Attri et al., (2000) and Verma and Hussain, (2013) in rats. Kamaraj et al., (2007) also found decrease in the activities of SOD, CAT and GST in lung cancer bearing animals.

Ramakrishnan et al., (2006) and Vinodh kumar et al., (2006) also found decreased activities of SOD and CAT in various carcinogenic and parasitic conditions. Significant increase of GST in groups A to F (few exceptions) in experimental groups of mice compared with that of Vinod Kumar and Viveka
Vardhani (2013) who found marked increase of GST in liver during *Ancylostoma caninum* infection. The decreased GST in groups A, B, C, D and E (on day 1-3 in A, on day 1 and 2 in B, on day 1 in C and E and on day 1 to 5 in D) might be due to the regulation of oxidative defense mechanism in the host. Sohail *et al.*, (2002) reported lowered GST activity during the malarial pathogenicity.

The observations of muscle fiber pathology and necrosis (in IDS+ vaccine treated animals) also confirm the onset of severe oxidative stress leading to tissue injury and ultimately disturbance in the synthesis of enzymes and proteins. The abnormal changes in the abdominal muscles of experimental groups may be due to oxidative stress and these observations compare well with the necroinflammatory changes of steatosis and necrosis (Sun *et al.*, 2002).

It is interesting to note that in groups I, A, B and C there was an increased AST activity when compared with the ALT activity. Increased AST activity signifies the increased transamination reaction within the muscle fibers. Also, in groups D, E and F, AST activity was found to be decreased when compared with ALT activity; this signifies the decreased transamination. Muthuviveganandave *et al.*, (2011) also related increased and decreased AST levels due to increased and decreased transamination in testes of male Swiss albino rats treated with a low dose of carbendazim (systemic fungicide). The significant alteration in the level of AST and ALT in all the experimental animals (when compared among the experimental groups) signifies the impact of varied doses of Gene Vac B vaccine on increased/decreased transamination process in muscle fibers.

The enhanced activity of ALP and ACP in all the experimental groups of animals (A, B, C, D, E and F) when compared with their counterpart controls and IDS treated animals confirms the dephosphorylation potential within the mouse muscle
fibers. The phosphate depletion may affect the ratio of calcium and phosphorus in hepatic cells which may eventually cause membrane damage and lack of energy compounds. Cells exposed to $\text{H}_2\text{O}_2$ (one of ROS), may generate OH which damages the cells that release alkaline phosphatase Mordente et al., (1987). The enhanced level of ACP in mice of groups A, B, C, D, E and F compare well with that of Halliwell and Gutteridge, (1990) who explained that under stress, acidosis and tissue damage, redox-active metal ions (copper or iron) will be liberated causing abnormality in metabolism and/or histology. The significant increase or decrease of ALP and ACP in the abdominal muscles of IDS treated + vaccinated animals suggests the adverse changes in the dephosphorylation of organic substances; this may be indirectly correlated with signal transduction events which occur in the hepatocytes of sensitized animals (Watts et al., 1995).

It is well known that hepatitis virus may cause abnormality in the metabolic function of various organs. Muscular dystrophy can be explained by the qualitative protein profile and quantitative studies of enzymes like AST, ALT, ALP, ACP, GST, SOD and CAT which are originally present in good quantity in abdominal muscles. The elevated levels of AST, ALT, ALP, ACP, GST and SOD and decreased level of CAT (with few exceptions) from day 1 to 5 in groups A, B, C, D, E and F correspond to the muscular dysfunction due to the invaded virus. Single dose of IDS treatment significantly altered the elevation of marker enzymes (higher than controls). These findings suggest the protective effect of IDS on muscle fibers in maintaining the natural stamina and energy. These results form a basis to conclude that IDS may be used as an adjuvant in immune mechanisms associated with muscular dystrophy; the synergistic activity of IDS (when given in pretreatment before the injection of Gene Vac B vaccine) is also confirmed. Marked alterations in the level of transaminases
(AST and ALT), phosphatases (ALP and ACP), GST, SOD and CAT in IDS treated, IDS treated + vaccinated mice reveal that these changes are not in accordance with the increasing doses of HBsAg. Statistical comparison among the experimental mice with regard to various enzymes also revealed significant and non significant levels.

The qualitative alterations of tissue proteins in the abdominal muscles may be due to the influence of stress proteins as suggested by Welch, (1993). The existence of common, additional and dominant polypeptides in the abdominal muscles of various groups of mice also confirm the influence of stress during viral infection. These results confirm that of Ghoshal et al., (1999, 2001) and Andrews, (2000) who also reported the synthesis of stress protein during viral infection/pathological conditions. The synthesis of stress protein is explained as a protective and repairing mechanism in vertebrates; the present gel electropherogram of muscles also showed marked qualitative alterations in protein fractions and profile due to IDS and/or HBsAg treatment. Das and Mukherjee, (2003); Begum, (2004) and Tarakalakshmi and Viveka Vardhani, (2014) reported the synthesis of stress protein in the liver of fish and in the small intestine of mice exposed to bacterial and nematode antigens.

Various changes were observed in the affected abdominal muscles of different experimental groups (A to F) of mice. These changes include: destruction, necrosis, infiltration, degeneration, cloudy swelling and loss of striation. Muscle fibers also showed inflammation and infiltration due to the influence of viral antigens although they were pretreated with the immunostimulant. These findings correlate with that of Sakunthala et al., (2014b) who found excessive secretion of mucus and slight to heavy necrosis in the gastric folds of the stomach of various groups of mice treated with IDS and varied doses of Gene Vac B vaccine. No significant changes were observed in the abdominal muscle fibers of immunostimulated mice (group I).
Though IDS treatment may cause slight stress leading to the production of ROS species thereby increasing SOD activity (Madhuri and Viveka Vardhani, 2014) in the abdominal muscles of IDS treated mice, IDS did not change the architecture of muscles. It is of interest to note that various doses of vaccine brought marked alteration in histology of abdominal muscles, though these changes were not dose dependent. Mice which received heavy doses (groups D to F) showed comparatively marked pathological changes compared to control, IDS treated and other experimentals (groups A to C). These observations are comparable to that of Munglang et al., (2009) and Zardozymy et al., (2010) who reported much histological changes in the hepatic organ during pesticide treatment. The present findings clearly confirm that of Sakunthala et al., (2014b) who also reported slight to heavy infiltration of cells and accumulation of cell debris in the lumen of stomach and large intestine of different groups of mice received IDS + varied doses of Gene Vac B vaccine.

The antioxidants in the IDS probably inhibited muscular dystrophy thereby increasing/decreasing the level of enzymes in IDS + vaccinated mice. IDS treatment alone markedly prevented the synthesis of enzymes and abnormal pathological reactions in the abdominal muscles and maintained near normalcy when compared to controls. This indicates the immunoprotective effect of IDS against viral antigens. The intake of diet along with immunostimulant may reduce the stress caused by antigens. However, further studies are required to understand the muscular dystrophy and/or enzymatic metabolism in mice treated with IDS alone and in those received IDS + HBsAg antigen.