CHAPTER IV
DISCUSSION

In the present *in vitro* study, it was found that addition of DEP significantly increases lipid peroxidation in mice liver and kidney homogenates. The increase was dose-dependent and almost similar in both liver and kidney homogenates as compared to controls (Table 3.1; Figure 3.1 & 3.2). Lipid peroxidation is a complex process known to occur in animals. It involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds is unsaturated lipids and the eventual distraction of membrane lipids, with the production of a variety of breakdown products (Dianzani and Barrera, 2008). Lipid peroxidation has been taken as an indicator of cellular oxidative damage (Halliwell *et al.*, 1999). The higher level of TBARS after DEP treatment was also reported in Wistar rat and olive flounder (*Paralichthys olivaceus*), a marine culture fish by Pereira *et al.* (2006) and Kang *et al.* (2010) respectively.

The protein content was significantly decreased in DEP - treated liver and kidney homogenates. The effect was dose-dependent (Table 3.2; Figure 3.3 & 3.4). This could be due to oxidative stress. Lipid peroxidation – derived free radical could attack back - bone of protein and side - chains of specific amino acid residues (Wu *et al.*, 2013) and might be responsible for significant reduction in protein content in DEP - treated liver and kidney homogenates.

The result of the present study revealed that DEP treatment for 30 days caused significant, dose-dependent reduction in body weight of mice (Table 3.4; Figure 3.5). The decrease in body weight is because of the reduced feed intake and anorexia which
was seen throughout the treatment period with LD, MD and HD of DEP. Brown et al. (1978) and Lamb et al. (1987) have reported significant (>10%) decrease in body weight and body weight gain in experimental animals after acute and intermediate – duration dietary exposure of DEP. Lamb et al. (1987) reported that dietary administration of DEP equivalent of 3250 mg/kg b.w./day was associated with 47% weight gain inhibition. A study by Brown et al. (1978) had explained that the reduction in body weight could be due to the lower food consumption and/or poorer food utilization.

Oral administration of DEP for 30 days in the present study caused increase in absolute as well as relative weights of liver and kidney of mice (Table 3.4; Figure 3.6 & 3.7). A significant increase (12%) in relative liver weight was observed in wistar rats, reported by Oishi and Hiraga et al. (1980). Brown et al. (1978) reported that administration of DEP had increased relative liver and kidney weights in both sexes of mice. This also agreed with Olsen et al. (1982). An increase in relative kidney weight was observed in male and female rats administered 5% diethyl phthalate in the diet (Brown et al., 1978). The marked increase seen in liver weight could be due to increased lipid and cholesterol content (Table 3.5), which has also been proved by histopathological investigations (Plate E) in the present study revealing fatty degenerative change (Plate C, D, E, H, I and J) in DEP - treated groups (Group 3, 4 and 5).

Treatment of all three doses of DEP caused significant increase in glycogen content in liver (Table 3.5; Figure 3.9). Liver is the main site for synthesis and storage of glycogen. Being a key organ involved in this metabolic process, increase in the glycogen level would indicate suppressed carbohydrate metabolism. Reduced
glycogen phosphorylase activity has been observed in liver of DEP - treated mice (Table 3.7). Accumulations of excessive glycogen due to DEP treatment could lead to hypertrophy of the hepatocytes, which could lead to complete impairment. Studies by Sonde et al. (2000) and Pereira et al. (2006) have also reported significant increase in liver glycogen content.

Diethyl phthalate administration caused significant increase in total lipid contents in liver and kidney of mice (Table 3.5; Figure 3.11). It might be due to inhibitory effect of DEP on acetyl-CoA carboxylase enzyme which is required to catalyse the rate-limiting step in fatty acid synthesis. The enzyme inhibition might be either direct by an alteration of the enzymes’ structure as a result of interaction between toxin and enzyme or indirect which may be through a toxin – induced changes in the cellular levels of allosteric effectors of acetyl-CoA carboxylase (Beynen and Geelen, 1982). PPAR-α (Peroxisome proliferator activated receptor alpha) is a transcription factor and a major regulator of lipid metabolism in liver and kidney, probably DEP exposure results in significant decrease in the β-oxidation process hence accumulation of fat or lipid occur in the major metabolic organ like liver and kidney (Mapuskar et al., 2006).

Table 3.5 shows increased level of cholesterol content in liver and kidney of mice. Synthesis and metabolism of cholesterol takes place mainly in liver following which it is transported to other organs. Highly significant increase in cholesterol level in liver and kidney indicates that DEP causes impairment in cholesterol metabolism. Some recent reviews on the role of StAR protein (Steroidogenic acute regulatory protein) of mitochondrial outer membrane indicate it as a regulator of cholesterol metabolism as well as steroidogenesis and cytochrome P450 side - chain cleavage
enzyme (P450scc), which is located on the matrix side of the inner mitochondrial membrane involved in transport of cholesterol and its metabolites for steroidogenesis (Nakae et al., 1997; Stocco et al., 2001). As per this we assume that DEP affects the StAR protein of mitochondrial outer membrane, hence accumulation of excessive cholesterol is observed. This results correlates well with earlier study in which male Sprague-Dawley rats administered with 50 ppm DEP through water shows in significant increase in liver cholesterol content (Sonde et al., 2000). Many other investigators (Mapuskar, 2007, Pereira, 2006) have reported increase cholesterol content in liver. Histopathological studies also revealed fatty accumulation in liver of DEP treated mice (Plates C, D and E).

Thirty days treatment of DEP in mice had resulted in significant, dose-dependent reductions in DNA, RNA and protein contents in the liver and kidney of mice (Table 3.5; Figure 3.12 & 3.13). DEP may affects biomarkers by forming DNA adducts (NAS/NRC 1989). NTP (1993) Board draft showed that DEP produced concentration-dependent increase in the number of relative sister chromatid exchange per chromosome in the presence of S9 fractions from rat liver homogenates. The positive sister chromatid exchange test might indicate a potential for DNA damage. Phthalates and/or their metabolites have been reported to damage DNA as assessed by the alkaline comet assay. Two recent epidemiologic reports involving men attending an infertility clinic showed that sperm DNA damage by DEP metabolite (Duty et al 2003; Hauser et al 2007). DEP is known to produce oxidative stress in the biological systems (Sun et al., 2012). Present in vivo and in vitro studies also reports significantly increased lipid peroxidation (Table 3.1, 3.2, 3.8 and 3.13). That could be the reason for the reduction in DNA, RNA and protein contents. Nucleic acids i.e. RNA and DNA are the major group of ROS targets. Oxidative DNA damage includes
modification of bases, DNA strand breaks and interstand crosslink (Miwa et al 2008).

DEP generated free radicals causes RNA damage could be due to incorporation of oxidized nucleotides during the process of RNA synthesis. DNA microarray analysis, followed by qrtPCR corroboration of the microarray data, demonstrated here that exposure of human cells to DEP in vitro is consistent with initiation of decreased expression of a large number of genes that have been identified as essential for fetal brain development (Hokanson et al., 2009). Reduction in nucleic acids also affects process of transcription and translation, ultimately causing reduction in tissue protein synthesis. Proteins are cellular targets of ROS (Starke-Reed and Oliver, 1989; Barnes et al., 2008). Oxidative modifications such as breakdown of peptide bonds, can damage protein structures (Stadtman, 2001) that related to the reduction of protein by elevated oxidative free radicals.

Oral administration of DEP to mice for 30 days had significantly altered the energy status. DEP treatment resulted in reduction in SDH activity – an enzyme bound to inner mitochondrial membrane, which could be due to structural and functional disorganization of the mitochondrial assembly (Fig .3.15). Srivastava et al. (1978 and 1977) reported that di (2-ethyl hexyl) phthalate (DEHP) also found to inhibit the activity of total and Mg⁺ - stimulated ATPase activity in rat liver. Beside liver, the activity of SDH and ATPse was also inhibited in rat heart, kidney (Srivastava et al., 1977), lung and gonads (Seth et al., 1976), indicating that suppression of energy- linked reactions may be a generalized effect of DEHP. Alteration in mitochondrial potential decreases the rate of cellular ATP synthesis and, thus nucleotide synthesis which may cause the reduction in DNA and RNA contents. Energy deficiency of the cell characterised by reduced activity of SDH and ATPase could be well correlated with reduction in protein content (Panet and Altan, 1979).
Mitochondria contains biochemical machinery for oxidation of various biomolecules and produced energy is captured in the form of ATP. Phthalates inhibited the respiration of isolated mitochondria from rat liver primarily by uncoupling oxidative phosphorylation (Inouye et al., 1978; Melnick et al., 1982). Other researchers have suggested that the phthalates inhibited electron transport or energy transport (Ohyama et al., 1976). Dibutyl phthalate and dimethyl phthalate inhibited the activities of SDH and ATPase, enzymes of the rat liver inner mitochondrial membrane (Srivastava et al., 1977; Tanaka et al., 1978; Melnick et al., 1982).

Diethyl phthalate treatment had significantly altered activities of liver marker enzymes (Table 3.16) indicative of hepatocellular membrane damage and necrosis. The present study revealed that ALT and AST activities significantly increased in DEP - treated animals as compared to controls. These two enzymes are localized normally within the cells of the liver, heart, kidney, gill, muscle and other organs (Wells et al., 1986). These enzymes are important markers in assessing and monitoring liver damage (Drotman et al., 1978). AST is a chief mitochondrial enzyme; damage to the mitochondria would result in leakage of this enzyme into the serum causing elevation in the serum enzyme levels, which were evident in all the treated groups. According to Gao et al. (2004), ALT activity is an important index to measure the degree of cell membrane damage, while AST is an indicator of mitochondrial damage since it contains 80% of this enzyme. Ghorpade et al. (2000) reported increased ALT and AST activities in liver and muscle of freshwater fish Cirrhina mrigala. Mapuskar et al. (2007) and Barse et al. (2007) also reported level of ALT and AST increased in mice, Cyprinus carpio respectively. These are of major importance in assessing and monitoring functional status of the liver.
Alkaline phosphatase (ALP) activity significantly and dose-dependently increased in serum of DEP-treated animals as compared to control and vehicle control. Alkaline phosphatase is a marker enzyme for plasma and endoplasmic reticulum (Wright et al., 1974; Shahjahan et al., 2004) is often employed to assess the integrity of plasma membrane (Akanji et al., 1993) Pereria et al. (2006). Ghorpade et al. (2000) and Barse et al. (2007) also reported increase ALP activity. Lipophilic DEP interact with plasma membrane and thereby could have led to increase in ALP activity (Barse et al., 2007).

Acid phosphatase (ACP) activity in its own case is a marker enzyme for the lysosomal integrity (Collins et al., 1971). The present study revealed significant, dose-dependent increase in ACP activity in serum of DEP-treated mice (Table 3.6). Similar changes were also reported by Mapuskar et al. (2007) in Swiss mice and Gorpade et al. (2000) in freshwater fish Cirrhina mrigala. It might be due to the consequence of damage to lysosomal integrity in the liver. The significant rise seen in the ACP activity after toxin administration may be attributed to an increase in cellular degeneration and other pathological liver injury (Verma et al., 2004).

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, some reactive oxidative species act as cellular messengers in redox signaling. Thus, oxidative stress can cause disruptions in normal mechanisms of cellular signaling. Oxidative stress is a condition in which the generation of reactive oxygen species (ROS), a ubiquitous by-products of aerobic
metabolism, overwhelm the cellular antioxidant defence mechanism. ROS have a crucial role in human physiological and pathophysiological process (D’ Autreaux et al., 2007).

Most xenobiotics damage liver and kidney by inducing, directly or indirectly lipid peroxidation. Lipid peroxidation is a major harmful consequences of reactive oxygen species (ROS) formation (Ambrosio et al., 1991; Lucas et al., 1998). Results shown in Table 3.8 & 3.13 indicate significant and dose-dependent increase in lipid peroxidation in liver and kidney of DEP-treated mice as compared to vehicle control. It could be due to either increased production of ROS by DEP and/or suppression of antioxidative defence system resulting in altered redox potential of cell causing lipid peroxidation and therefore, suggesting a considerable oxidative stress. Pereira et al. (2006) have correlated increased lipid peroxidation levels with the increased number of peroxisomes which indicates high level of ROS production due to continuous liver and kidney insult by DEP.

Increased lipid peroxidation could also be due to impairment in enzymatic and non-enzymatic antioxidant system in the tissues. Total glutathione (GSH) and total ascorbic acid (TAA) contents are important endogenous free radical scavenger and non-enzymatic antioxidants. Oral administration of DEP for 30 days has resulted significant reduction in level of GSH and TAA contents (Table 3.8 & 3.13). Glutathione functions as an electrophile, radical scavenger and a redox partener (Meister et al. 1985). This reduction would result in increased free radical injury in tissue leading to extensive tissue damage. Pereira et al. (2006) had shown reduction in GSH content in DEP exposed wistar rats which support our study. During free radical scavenging action, ascorbic acid, transformed into L-dehydroascorbate back to
ascorbate (Breimer, 1990). The fall in the level of reduced glutathione decreases conversion of L-dehydroascorbate and probably explains the lowered level of TAA in DEP- treated animals.

Activities of enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione transferase) were found to reduce with DEP treatment for 30 days (Table 3.9; Figure 3.20 and 21), which could be due to increased production of free radicals characterized by increased MDA content. These enzymes are known to scavenge free radicals such as superoxide, hydroxyl and hydrogen peroxide, thus preventing damage caused by oxidative stress to the tissue. Superoxide radicals have been reported in several pathological disorders. A reduction in SOD activity contributes to increasing the level of superoxide radicals, thus leading to increased oxidative stress which enhances early cell death. Pereira et al. (2006) also reported depletion of GSH and GR in female Wistar rats. Diethyl phthalate failed to up-regulate expression of mRNA of the enzymes SOD, CAT, GST which are induced as a counter measures to the adverse effects of oxidative stress. These data corroborate recent reports in the literature (Rosado-Berrios et al., 2011; Erkekoglu et al. 2011, 2012) and suggest that oxidative stress is a critical mechanism of toxicity for DEP.

Dietary antioxidant intake may be an important strategy for inhibiting or delaying the oxidation of susceptible cellular substrates, and is thus relevant to disease prevention in many paradigms. Plants, including herbs and spices, have many phytochemicals which are potential sources of natural antioxidants. Phenolic compounds such as flavonoids, phenolic acids and tannins have received attention for their high antioxidative activity (Rice – Evans et al., 1996). In the present study qualitative assessment of phytochemicals of hydro-alcoholic seed extract of Nigella
Nigella sativa showed presence of phenols, flavonoids and tannins. Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts (Wolfe et al., 2003; Naik et al., 2006). The antioxidative property of polyphenols is a predominant feature of their radical-scavenging capacity (Facino et al., 1990; Yang et al., 2001; Cotelle et al., 2001). In the present study quantitative estimation of crude polyphenols from hydro-alcoholic seed extract of Nigella sativa revealed presence of significantly high amount of phytochemicals principally responsible for its protective effect (Table 3.16). Flavonoids are 15- carbon compounds generally distributed throughout the plant kingdom (Harborne 1988). Flavonoids and many other phenolic compounds of plant origin have been reported as scavengers of ROS and viewed as promising therapeutic drugs for free radical pathologies (Prashad et al., 1998; Chang et al., 2007). Tannins are the most abundant antioxidants in the human diet and they exhibit many biologically important functions which include protection against oxidative stress (Atanassova and Christova-Bagdassarian, 2009). The oxidation inhibiting activities of tannins have been known for long time (Edeoga et al., 2006).

Hydro-alcoholic Nigella sativa seed extract was found to be potent scavenger of superoxide, hydroxyl, nitrous oxide and DPPH radicals (Figure 3.41, 3.42, 3.43 and 3.44). Free radical scavenging effect of Nigella sativa had also been reported by Meziti et al., (2012). These scavenging properties are generally due to high reducing capacity of polyphenols acting as primary antioxidants (Javanovic et al., 1999). Nigella sativa is powerful scavenger of DPPH and hydroxyl radical (Burits et al., 2000). Meziti et al. (2012) reported significant linear correlation between their superoxide anion scavenging effect and their phenolics and flavonoid contents. Table
3.16 showed presence of correlation between total phenolic content and antioxidant effect of hydro-alcoholic extract of *Nigella sativa* seed.

*Nigella sativa* seed extract alone did not have any significant effect on morphological changes, change in body weight as well as absolute and relative weights of liver and kidney as compared to untreated and vehicle control. Table 3.19 shows the mitigatory effect of two different doses of *Nigella sativa* seed extract on DEP induced body weight reduction. Maximum toxicity had been exerted in high dose of DEP; cotreatment of *Nigella sativa* seed extract along with high of DEP resulted in significant amelioration in body weight of animals. This protective effect might be due to reduced oxidative stress levels in *Nigella sativa* seed extract treated animals resulting in normalization of food intake and metabolism. Kaleem *et al* (2006) have shown protective effect of *Nigella sativa* seed extract on body weight in diabetic rats. The result indicated reduction in animal body weight under oxidative stress condition which was successfully ameliorated with cotreatment of two different dosage of *Nigella sativa* seed extract (Kaleem *et al.*, 2006).

Oral administration of DEP had significantly increased the absolute and relative weight of mice liver and kidney. Table 3.20 shows that oral administration of hydro alcoholic extract of *Nigella sativa* seed in two different doses significantly combated these DEP induced changes; maximum effect was observed with high dose of *Nigella sativa* seed extract. This protective effect might be due to lipid lowering effect of the hydro – alcoholic extract of the *Nigella sativa* seed. Histopathological studies revealed reduced fatty infiltration in DEP plus *Nigella sativa* seed extract treated liver and kidney of mice (Plate M and P). Supplementation of *Nigella sativa* seed extract resulted in lowering of the total lipid, cholesterol, triglycerides in the
albino rats (Badary et al., 2000; El Dakha khani et al. 2000; Le et al., 2004; Bhatti et al., 2009).

Diethyl phthalate treatment for 30 days had significantly increased lipid and cholesterol contents in liver and kidney of mice (Table 3.21). Combined treatment of Nigella sativa seed extract and DEP caused mitigation in DEP caused alteration in lipid and cholesterol contents in liver and kidney of mice. The lipid lowering effect of Nigella sativa seed extract might be due to its phytochemicals which are present in it. Nigella sativa seed had favourably modified serum lipid profile in rats with significant decrease in total cholesterol, LDL-cholesterol and triglycerides (Al-L-Ogmani et al., 2011). Kocyigit et al. (2009) reported that Nigella sativa seed to diet is a remedy that may prove to be useful in the prevention and treatment of hyperlipidemia and hypercholesterolemia. This protective effect could be due to presence of some active ingredients like, saponins which helps in improving serum lipid profile and tissue lipid and cholesterol contents.

Nigella sativa seed extract alone treatment did not show any significant effect on protein and glycogen contents of mice. Oral administration of Nigella sativa seed extract with high dose of DEP caused significant mitigation in DEP caused alteration in protein and glycogen contents. Alsaif et al. (2007) had reported that Nigella sativa treatment in ethanol- induced rat increase the total protein level which is in accordance with our study. Nigella sativa seed decrease the glycogen content in 12 week treated rats (Zaoui et al., 2002).

Diethyl phthalate treatment for 30 days caused significant reduction in nucleic acid and protein content in liver and kidney of mice which might be due to oxidative damage. Oxidative DNA damage might be recovered by the antioxidants which can either stimulate the act of repair enzyme or directly protect against oxidation.
Nigella sativa seed extract is excellent antioxidants and thus prevents the effect of oxidative stress. The present study shows that cotreatment of Nigella sativa seed extract along with high dose of DEP significantly mitigated these changes majorly due to its antioxidative potency. Nigella sativa decreased the DNA breakage, which may be attributed to its ROS scavenging activity or upregulation of DNA repair genes which protects against radiation – induced damage by bringing error free repair of DNA damage (Pereira et al., 2012). Parveen et al., (2011) reported that Nigella sativa reduced the number of gaps, breaks, exchange, dicentrics and rings in the chromatid type aberration and chromosome type aberration in bone marrow cells of rats. Nigella sativa seed contain proteins, alkaloids, saponin and has been found to increase the level of total protein through stimulation of protein synthesis (Salem et al., 2005). Besides, Nigella sativa causes suppression of gluconeogenesis and prevents catabolism of protein and conversion to glucose (Al-Gaby et al., 1998), and this may lead to increase level of total serum protein concentration. Oxidative RNA damage is also a feature in xenobiotic –induced toxicities suggesting that RNA oxidation may actively contribute to the onset or to the development of disease (Nunomura et al., 2006). DNA and RNA are main constituents for protein synthesis. Therefore, elevation of protein content was just because of restored levels of DNA and RNA content by Nigella sativa seed extract which normalises the process of transcription and translation.

Hepatotoxicity of DEP was evaluated by estimating the activities of various liver marker enzymes in tissue and serum. As indicated in Table 3.6 activities of ALT, AST, ALP and ACP were found to increase with DEP treatment in liver tissue as well as serum of mice. The present result is in agreement with Daba and Abdel Rahman (1998) who showed, Nigella sativa protective effect against tertiary-butyl
hydroperoxide induced hepatotoxicity in rats isolated hepatocytes. Our findings reveal that administrations of *Nigella sativa* significantly restores back the levels of liver marker enzymes (Table 3.22). *Nigella sativa* treatment protected mice against hepatotoxicity induced by CCL\(_4\) (Badary *et al.*, 1997). *Nigella sativa* seeds restored the enzymes level in carbon tetrachloride –induced hepatotoxicity in rats (Iihan *et al.*, 2005). Gani *et al.* (2013) showed *Nigella sativa* extract being able to protect liver tissues and decrease the leakage of the enzymes (ALT, AST, ALP and ACP) into the circulations.

All three doses of DEP were found to reduce the activities of SDH and ATPase activities in liver and kidney of the animals resulting in altered status. Treatment with *Nigella sativa* seed extract along with DEP significantly ameliorates DEP caused changes in the activities of SDH and ATPase in liver and kidney of mice (Table 3.23). Erşahin *et al.* (2011) reported that *Nigella sativa* with its potent free radical scavenging properties, inhibited subarachnoid-haemorrhage-(SAH-) induced lipid peroxidation in the brain tissue of rat against the reactive hydroxyl, peroxyl, and superoxide radicals. In addition, the level of antioxidant glutathione (GSH) was preserved, thereby ameliorating oxidative damage. The SAH-induced reduction of Na\(^+/\)K\(^+\)-ATPase activity indicated the presence of membrane damage. The Na\(^+/\)K\(^+\)-ATPase is involved in the generation of the membrane potential through the active transport of sodium and potassium ions in cellular membrane. It maintains neuronal excitability and controls cellular volume in the central nervous system. Treatment with *Nigella sativa* was able to restore Na\(^+/\)K\(^+\)-ATPase activity back to normal levels. Hamed *et al.* (2013) reported that treatment with black seed alleviated the elevation of SDH and Na\(^+/\)k\(^+\) ATPase. The restoration of ATPase activity suggest the ability of *Nigella sativa* to protect the sulphhydryl group from oxidative damage through
inhibition of lipid peroxidation. Normalised metabolism of protein, carbohydrates and lipid as well as free radical scavenging effect of plant improves integrity and oxidative phosphorylation in mitochondria which was highly disturbed in case of energy deficient state-induced by DEP.

Diethyl phthalate treatment in mice elevated levels of lipid peroxidation in liver and kidney which was highest with the administration of high dose of DEP (Table 3.8 & 3.13). Cotreatment of Nigella saiva seed extract along with high dose of DEP cause significantly reduced levels of LPO as compared to DEP alone treated animals which might be due to antioxidative and free radical scavenging activity of Nigella sativa polyphenols as it was well correlated in our in-vitro studies (Table 3.16). The reduced lipid peroxidation level in liver and kidney of Nigella sativa plus DEP – treated mice could be due to significant elevation in enzymatic and non-enzymatic antioxidants as well as antioxidative properties of Nigella sativa seed extract itself. Nigella sativa decreases the lipid peroxidation and increases the antioxidant defence system activity in the CCL₄ treated mice (Kanter et al. 2003). Nigella sativa seed extract increases GSH and TAA contents in DEP intoxicated animals (Table 3.24 & 3.29) which could be due to antioxidative properties. Administration of Nigella sativa restored the activities of nonenzymatic (GSH) and enzymatic (SOD, CAT, GPx, and glutathione-S-transferase GST) antioxidants as well as reduced the levels of malondialdehyde (MDA) in the rat brain to normal levels (Sheikh et al., 2012). Kaleem et al. (2006) reported that oral administration of ethanol extract of Nigella sativa seeds (300 mg/kg body weight/day) to streptozotocin-induced diabetic rats for 30 days significantly reduced the elevated lipid and improved altered levels of lipid peroxidation products (TBARS and hydroperoxidase) and antioxidant enzymes like catalase, superoxide dismutase, reduced glutathione and
glutathione peroxidase in liver and kidney. Hydro-alcoholic extract of *Nigella sativa* seed is an excellent scavenger of superoxide, hydroxyl, nitrous oxide and DPPH radical as indicated in our *in-vitro* studies. This could be the reason for *Nigella sativa* seed extract–induced increase in enzymatic antioxidants.

*Nigella sativa* seed extract-treatment did not show any significant effect on histopathological changes in liver and kidney of mice as compared to untreated and vehicle control. Histopathological studies revealed increased in vacuolization, fatty infiltration and hepatocellular necrosis in the liver of DEP–treated mice (Plate E). In kidney the histopathological studies revealed degenerative changes, increase vacuolization, disorganization of glomerulus and increased space between the glomerulus and the capsule wall in DEP-treated mice (Plate J). This might be due to the significant reduction in enzymatic and non-enzymatic antioxidants and increased malondialdehyde (MDA) induced oxidative damage to liver and kidney. Ikele *et al.* (2011) also reported histopathological changes in liver and kidney of DEP-treated *Clarias gariepinus*. However, co-treatment of *Nigella sativa* seed extract along with DEP caused alleviation in diethyl phthalate caused alteration in liver (Plate M) and kidney (Plate P) of mice. This might be due to antioxidative properties of *Nigella sativa* seed extract as results in Table 3.16 shows that conjugate treatment of *Nigella sativa* seed extract and DEP decreases lipid peroxidation and increases activities of antioxidant enzymes. *Nigella sativa* seeds showed no significant changes in the hepatocytes of rats (Al-Okbi *et al.*, 1997) and in liver and kidney functions (Ali and Blundes, 2003). The preventive activity of *Nigella sativa* may related to its antioxidants efficiency that inhibit lipid peroxidation (Farrag *et al.*, 2007). *Nigella sativa* showed protective effect against lead acetate–induced hepatic tissue damage in mice (Alarifiet *et al.*, 2012). Aziz Dollah *et al.* (2012) reported supplementation of
*Nigella sativa* seed to the diets of rats for five weeks did not change the biochemical parameters of kidney functions as well as histopathological investigation which illustrated normal architecture of kidney (Le et al., 2004).

Increased hepatocellular damage as observed in the histopathology could be due to oxidative damage leading to increased free radicals which well correlates with the increased liver marker enzymes in serum like, ALT, AST, ALP and ACP. It is also reported by Pereia et al. (2006) in serum DEP-treated rats. Elevated levels of serum enzymes are indicators of cellular leakage and loss of functional integrity of the cell membrane in liver (Orisakwe et al., 2003). Thus, their increased presence in serum may give information on organ dysfunction (Wada et al., 1962). There is no significant changes in *Nigella sativa* alone treated animals. Cotreatment of *Nigella sativa* seed extract along with high dose of DEP decrease the activities of ALT, AST, ALP and ACP levels (Table 3.31). Nehar et al. (2012) reported that *Nigella sativa* alleviated the increased level of ALT, AST, ALP to the near normal which could be manifested to reduction in cell membrane disturbance.

Diethyl phthalate also decreased the protein as well as cholesterol contents in the serum (Table 3.15). No significant changes were observed in all the control groups as well as *Nigella sativa* seed extract alone treatment. Impaired cholesterol transport was evident in the higher dose treated groups due to reduced levels in the serum cholesterol level. The serum concentration of creatinine is relatively constant under normal circumstances, unless glomerular filtration rate (GSR) changes, as a result of defective renal function. Serum creatinine was examined as indicator for kidney function. Oral administration of DEP for 30 days caused elevation in serum creatinine content. The increased level of serum creatinine after DEP intoxication (Table 3.31) might be due to reduced ability of the kidney to eliminate the toxic metabolic
substances (Kummer et al., 1988). However, cotreatment of *Nigella sativa* along with high dose of DEP leads to prevents the elevation to serum creatinine in kidney of mice. Al-Okbi *et al.* (1997) reported that oral administration of *Nigella sativa* seeds and extracts caused significant reduction in serum creatinine content.