CHAPTER III

RESULTS

The present investigation was aimed to assess the toxic effects of DEP in both *in vitro* and *in vivo* conditions and its amelioration by *Nigella sativa* seed extract. The whole study was divided in three parts: **Part I:** To study the toxic effects of diethyl phthalate both *in vitro* and *in vivo* conditions. **Part II:** Phytochemical analysis of the *Nigella sativa* seed extract. **Part III:** To study the ameliorative effect of *Nigella sativa* seed extract on toxicity induced by diethyl phthalate.

**PART I**

*To study the toxic effects of diethyl phthalate both in vitro and in vivo conditions*

*In vitro study*

Table 3.1 and 3.2 indicates the effect of DEP on production of thiobarbituric acid reactive substances (TBARS) in liver and kidney homogenates. Addition of DEP (10-40 µg/mL) to liver and kidney homogenates significantly (p<0.05) increased H₂O₂-induced lipid peroxidation *in vitro*. The effect was found to be concentration – dependent (r²=0.993 and 0.904 respectively) (Fig 3.1 and 3.2). At 40 µg/mL DEP concentration maximum TBARS production was found in both liver and kidney homogenates.

Similarly, effect of DEP treatment on protein content was evaluated *in vitro* condition in liver and kidney homogenates. Results revealed that protein content
decreased as the concentration of DEP was increased in liver and kidney homogenates (Table 3.1 and 3.2). The effect was significant (p<0.05) and dose-dependent ($r^2 = 0.849$ and 0.948 respectively). Maximum reduction in protein content was observed at 40 µg/mL of DEP (Fig. 3.3 and 3.4).

**In vivo study**

**Clinical observations**

No treatment related clinical signs were observed in control animals (Group 1 and 2). DEP treatment for 30 days caused dullness and lethargy (Groups 3, 4 and 5).

**Body weight**

There was no significant difference in the average initial body weight of different groups of animals. At the end of 30 days treatment, no significant change in body weight was observed between different control groups of mice (Groups 1 and 2). As compared with vehicle control, oral – administration of DEP caused reduction in body weight (Groups 3, 4 and 5) (Table 3.3). The effect was significant (p<0.05) and dose-dependent ($r^2 = 0.939$). As shown in Figure 3.5 oral administration of LD, MD and HD of DEP had reduced body weight by 8.81%, 11.95% and 15.79% respectively as compared to vehicle control.

**LIVER:**

**Absolute and relative liver weights**

No significant alteration was observed in the absolute and relative liver weights of group 1 and group 2 animals (untreated and vehicle control). The oral administration on DEP for 30 days caused significant (p<0.05) increase in absolute and relative liver weights as compared to vehicle control (Table 3.4). The effects were dose-dependent ($r^2 = 0.981$, 0.973 respectively). As compared to vehicle control, oral
administration of LD, MD and HD of DEP had increased absolute liver weight by 32.61%, 41.24% and 67.86% respectively (Figure 3.6). Similarly significant (p<0.05) increase in relative weight was observed in DEP- treated animals (LD: 18.84%, MD: 29.71% and HD: 41.30%; Figure 3.7)

**BIOCHEMICAL ANALYSIS**

**Protein, carbohydrate, lipid and nucleic acid contents:**

**Protein content:**

Table 3.5 shows the effect of DEP treatment on protein content in the liver of mice. No significant change in protein content was observed between untreated and vehicle control groups (Groups 1 and 2). Protein content in liver of all three doses of DEP – treated animals were reduced significantly (p< 0.05), as compared with vehicle control animals. The reduction in protein content by 30 days oral treatment of DEP (LD:17.73% , MD:37.73% and HD:51.20%; Figure 3.8 ) was dose-dependent ($r^2 = 0.994$).

**Glycogen content:**

Glycogen content in liver of DEP – treated animals were observed to increased significantly (p<0.05) when compared to animals of control groups (Table 3.5). All three doses of DEP (LD: 10.94%, MD: 22.63% and HD: 37.10%) had dose-dependently ($r^2 = 0.995$) the content of glycogen as compared to control (Figure 3.9). No significant difference in glycogen content was observed in control animals.
**Cholesterol content:**

Diethyl phthalate treatment for 30 days caused increase in hepatic cholesterol content as shown in Table 3.5. No significant difference was noted between cholesterol content of untreated and vehicle control groups. Oral administration of DEP with three different doses increased cholesterol content dose-dependently ($r^2 = 0.932$). However, significant ($p<0.05$) increase was only in high dose of DEP-treated animal. Percent increase in cholesterol content by LD, MD and HD was 14.01%, 30.84% and 71.02% respectively (Figure 3.10).

**Total lipid content:**

Table 3.5 shows the effect of DEP treatment on total lipid content in the liver of mice. Results revealed that oral treatment of DEP caused dose-dependent ($r^2 = 0.976$) increase in total lipid content. However, significant increase was only in mid and high dose treated animals. Oral administration of LD, MD and HD of DEP had increased total lipid content by 14.32%, 39.25% and 69.62% respectively (Figure 3.11) as compared to vehicle control. No significant increase in total lipid content was observed between untreated and vehicle control groups.

**DNA content:**

DNA content in liver of group 1 and 2 (untreated and vehicle control) animals showed no significant changes. On the contrary, oral administration of DEP for 30 days significantly ($p<0.05$) and dose-dependently ($r^2 = 0.958$) decreased DNA content in liver of mice. LD, MD and HD of DEP (group 3, 4 and 5) reduced DNA content by 7.19%, 23.63% and 45.70 respectively (Table 3.5; Figure 3.12)
**RNA content:**

Table 3.5 showed the reduction in RNA content in liver upon DEP treatment. Different doses of DEP had significantly (p<0.05) reduced RNA content as compared to vehicle control. The effect was dose-dependent ($r^2=0.983$) and was highest with HD treatment (LD: 9.53%, MD: 19.00%, HD: 24.16%; Figure 3.13).

**ENZYMATIC ANALYSIS:**

**Activities of ALT, AST, ACP and ALP in liver:**

Table 3.6 shows the effect of oral administration of DEP on activities of ALT, AST, ACP and ALP in liver of mice. There were no significant alteration in the activities of ALT, AST, ACP and ALP in untreated and vehicle control animals (group 1 and 2). On the contrary, DEP treatment for 30 days had significantly (p<0.05) increased the activities of ALT, AST, ACP and ALP in a dose-dependent manner ($r^2= 0.998, 0.991, 0.979$ and $0.998$ respectively). Oral treatment of LD, MD and HD of DEP (group 3, 4 and 5) increased ALT activity by 35.38%, 76.15% and 116.92% respectively (Figure 3.14). Similarly, increase in AST activity by DEP treatment was up to 99.61% (LD-14.61%, MD-49.29% and HD-99.61%; Figure 3.14). Maximum percent increase in the activities of ACP and ALP was with high dose of DEP [(LD: 54.50%, 72.72; MD: 86.50%, 142.42%; HD: 158.50%, 224.24%) respectively (Figure 3.14)].

**Enzymes involved in energy metabolism:**

Table 3.7 depicts the results of various doses of DEP caused changes in energy metabolism. No significant changes were observed in the activities of SDH and ATPase in the liver of untreated and vehicle control group (group 1 and 2). However,
in all three doses of DEP treatment (group 3, 4 and 5) significant (p<0.05) reduction was observed in the activity of SDH (LD: 23.38%, MD: 42.68%, HD: 51.30%) (Figure 3.15). Similarly, Figure 3.16 shows reduction in activity of ATPase (LD: 12.99%, MD: 40.26%, HD: 67.54%) as compared to group 2 animals (vehicle control). However, significant reduction was in mid and high doses DEP-treated groups. The reduction in both SDH and ATPase activities were dose-dependent ($r^2$=0.963 and 0.977 respectively).

**Phosphorylase activity:**

No significant changes were observed in phosphorylase activity in liver of control groups (group 1 and 2). Oral administration of three different doses (LD, MD and HD) of DEP caused decrease phosphorylase activity significantly (p<0.05) and dose – dependently ($r^2$= 0.985) in liver (16.06%, 35.04% and 62.05% respectively) (Table 3.7; Figure 3.17)

**Lipid peroxidation and non-enzymatic and enzymatic antioxidants:**

Table 3.8 shows the effects of DEP treatment on lipid peroxidation and non-enzymatic antioxidants (GSH and TAA) in liver of mice. No significant changes were noted in LPO, GSH and TAA contents between different control groups (group 1 and 2). Administration of various doses of DEP had significantly altered the level of lipid peroxidation and non-enzymatic antioxidants (GSH and TAA). As compared to vehicle control, treatment of LD, MD and HD of DEP increased lipid peroxidation significantly (p<0.05) and dose-dependently ($r^2$= 0.946) in liver (27.75%, 55.02% and 118.66% respectively) (Figure 3.18). Oral treatment of DEP for 30 days significantly (p<0.05) reduced level of non-enzymatic antioxidants (GSH and TAA) as compared to vehicle control (group 2). Percent reduction in GSH content by LD:13.17%,

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MD: 32.09% and HD: 53.24% of DEP was in a dose-dependent manner ($r^2 = 0.989$). Similarly, depletion of TAA content (LD: 18.57%, MD: 30.74%, HD: 52.27%) was significant (p<0.05) and dose-dependent ($r^2 = 0.989$) and highest with HD treatment (Figure 3.19).

Administration of various doses of DEP had significantly altered the levels of enzymatic antioxidants (CAT, SOD, GR, GST and GPx) in liver of mice. Table 3.9 represents the effect of DEP on CAT, SOD, GR, GST and GPx activities in liver of mice where no significant alteration was observed between untreated and vehicle control groups. As shown in Table 3.9, treatment of LD, MD and HD of DEP decreased activities of CAT and SOD significantly (p<0.05) and dose-dependently ($r^2 = 0.960, 0.944$ respectively) in mice liver (LD: 11.18%, 25.46%; MD: 31.83%, 50.91%; HD: 63.29%, 69.10% respectively; Figure 3.20) as compared to vehicle control (group 2). Activities of GR, GST and GPx were found to reduce significantly (p<0.05) and dose-dependently ($r^2 = 0.946, 0.931$ and 0.942 respectively). Reduction in GR activity by LD, MD and HD of DEP was by 19.37%, 35.88% and 40.96% respectively (Figure 3.21). Figure 3.21 represents percent decrease in the activities of GST (LD: 4.47%, MD: 41.97%, HD: 64.52%) and GPx (LD: 8.05%, MD: 51.55%, HD: 73.22%).

**HISTOPATHOLOGICAL STUDY:**

Light microscopic study of liver histopathology of untreated control (Group 1) mice showed normal morphology, size and histo-architecture and liver tissue (Plate A). Plate B. shows that oral treatment of olive oil (Group 2) did not cause any undesirable changes in hepatocytes structure and liver histology. Treatment with low dose (310 mg/ kg b.w./day) (Plate C) and mid dose (620 mg/ kg b.w./ day) (Plate D)
of DEP caused mild to moderate damage in hepatocytes, fat deposition and slightly showed intracellular vacuolation in mid dose. On the other hand, oral administration of high dose (1240 mg/ kg b.w./day) of DEP for 30 days caused severe intracellular vacuolation, fat deposition and loss of hepatic architecture as compared to control. Most pronounced toxic effect of DEP was observed with high dose treatment which was visible in hematoxylin-eosin stained histopathological slides as shown in Plate E.

**KIDNEY**

**Absolute and relative weights**

Table 3.10 shows changes in absolute and relative weight of kidney in various doses of DEP – treated mice. Percent changes in absolute and relative weights of kidney are shown in Figures 3.22 and 3.23 respectively. Different control groups (group 1 and 2) had non-significant changes in absolute kidney weight. Data shown in Table 3.10 indicates that DEP – treatment for 30 days caused significant (p<0.05) only in MD and HD and dose-dependent \( (r^2=0.994) \) increase in absolute kidney weight \[LD: 15.38\%\, MD: 25.64\%\, and HD: 38.64\% \] (Figure 3.22) as compared to vehicle control (group 2).

There was no significant change in relative kidney weight between different control groups (group 1 and 2). As compared with vehicle control (group 2), oral administration of DEP for 30 days caused, significant (p<0.05) only in MD and HD, dose-dependent \( (r^2= 0.990) \) increase \[ (LD-27.35\%,\, MD-43.58\%\, and HD-64.95\% \] (Figure 3.23) in relative kidney weight in mice (Table 3.10).
**BIOCHEMICAL ANALYSIS**

*Protein, lipid and nucleic acid contents:*

**Protein content:**

Protein content in kidney of DEP – treated animals decreased significantly (p<0.05) when compared to vehicle control group (Table 3.11). No significant reduction in protein content of untreated and vehicle control groups was observed. Figure 3.24 shows that the reduction in protein content by oral treatment of DEP (LD: 12.82%, MD: 31.33% and HD: 54.15%) was dose-dependent (r²=0.984).

**Total lipid content:**

DEP treatment for 30 days caused significant (p<0.05) elevation in lipid content as shown in Table 3.11 All three doses of toxin (LD: 24.91%, MD: 59.78% and HD: 89.32%) dose-dependently (r²=0.996) increased total lipid content (Figure 3.25) as compared to vehicle control. Lipid content of untreated and vehicle control group were almost similar.

**Cholesterol content:**

Cholesterol content of animals of group 1 and 2 (untreated and vehicle control) showed no significant changes. However, oral administration of DEP for 30 days significantly (p<0.05); only MD and HD and dose-dependently (r²= 0.897) increased cholesterol content. Percent increase in cholesterol content by LD, MD and HD was 24.64%, 73.94% and 170.42% respectively (Table 3.11; Figure 3.26).
**DNA content:**

Table 3.11 showed the reduction in DNA content in kidney tissue upon DEP treatment. Different doses of DEP had significantly (p<0.05) reduced DNA content as compared to untreated and vehicle control. The effect was dose-dependent (r²=0.886) and was highest with HD treatment (LD-6.29%, MD-19.17% and HD-51.79%; Figure 3.27).

**RNA content:**

Diethyl phthalate was found to reduce RNA content of kidney significantly (p<0.05) and dose-dependently (r²=0.962). As shown in Table 3.11, no significant difference was noted between RNA content of untreated and vehicle control groups. Treatment with LD, MD and HD of DEP (group 3, 4 and 5) reduced RNA content by 7.47%, 33.34% and 48.68% respectively (Figure 3.28).

**ENZYMATIC ALTERATIONS:**

**Enzymes involved in energy metabolism:**

Table 3.12 represents effect of various doses of DEP on activities of SDH and ATPase. No significant change in the activities of SDH and ATPase of untreated and vehicle control group was observed. As shown in Figure 3.29, LD, MD and HD of DEP treatment reduced SDH activity in kidney by 16.00%, 28.77% and 45.75% respectively. The reduction was significant (p<0.05) as compared to control (group 1 and 2) and was dose dependent (r²=0.998). The DEP treatment to the animals of groups 3, 4 and 5 (LD-15.88%, MD-28.58% and HD-48.42%) reduced the activity of
ATPase (Figure 3.30). The effect was significant (p<0.05) and dose-dependent (r² = 0.992) as compared to control group animals.

**Activity of ACP in kidney:**

Results revealed that no significant changes were observed between different control groups (group 1 and 2). However, DEP treatment for 30 days had significantly (p<0.05) increased the activity of ACP in a dose-dependent manner (r² = 0.991). Percent increase in the activity of ACP by LD, MD and HD was 37.96%, 92.59% and 150.00% respectively (Table 3.12; Figure 3.31).

**Lipid peroxidation and non-enzymatic and enzymatic antioxidants:**

Table 3.13 represents the effect of DEP on lipid peroxidation level and non-enzymatic antioxidants (GSH and TAA) in the kidney of mice where no significant alteration was observed between untreated and vehicle control group. Oral administration of DEP increased lipid peroxidation significantly (p<0.05) and dose-dependently (r² = 0.820) in mice kidney (LD: 27.12%, MD: 31.54% and HD: 116.71% respectively; Figure 3.32). Also treatment of DEP for 30 days significantly (p<0.05) reduced level of non-enzymatic antioxidants (GSH and TAA) as compared to control (group 2). Percent reductions in GSH and TAA contents by DEP treatment were dose-dependent [LD:14.49%, 9.98%; MD:29.48%, 21.94%; HD:45.49%, 51.19% respectively; (r² = 0.999, 0.929 respectively)] (Figure 3.33).

The results of enzymatic antioxidative defence parameters in kidney of DEP - treated mice is shown in Table 3.14. No significant difference in activities of CAT, SOD, GR, GST and GPx were observed between different control groups (group 1
and 2). As compared to vehicle control group, DEP treatment for 30 days caused significant (p<0.05) decrease in activities of CAT (LD:16.67%, 31.79%, HD:53.99%) and SOD (LD:34.98%, 60.10%, HD:71.43%) in a dose-dependent manner (r²=0.992, 0.953 respectively) (Figure 3.34). Similarly, Figure 3.35 shows reduction in activities of GR (LD:17.86%, 39.55%, HD:54.09%), GST (LD:16.67%, 31.79%, HD:53.99%) and GPx (LD:22.90%, 57.41%, HD:72.56%) by DEP treatment. The effect was dose-dependent (GR-r²= 0.994, GST- r²= 0.941 and GPx - r²= 0.980).

**HISTOPATHOLOGICAL STUDY**

The transverse section of kidney of vehicle and untreated control mice showed normal Bowman’s capsule with glomerulus as well as proximal and distal convoluted tubules (Group 1 and 2) (Plate F and G). Oral administration of DEP for 30 days caused distortion of the tubules, increased vacuolization, necrosis, atrophy of the glomerulus and increased space between glomerulus and capsule wall (Groups 3, 4 and 5) (Plate H, I and J). The effect was more prominent in high dose DEP-treated animals than that of low dose.

**Serum parameters**

Table 3.15 shows the effect of DEP treatment on serum parameters. Oral administration of three different doses of DEP increased activities of ALT, AST, ACP, ALP and creatinine and glucose contents as well as decreased protein and cholesterol contents significantly (p<0.05). In serum dose-dependent increase was found in the activity of ALT (r²= 0.918) and AST (r²= 0.945). The rise in case of serum ALT activity by LD, MD and HD of DEP was 31.76%, 147.05% and 323.52% which was 14.61%, 49.29% and 99.61% in case of AST activity. DEP – induced
elevation in activity of ACP and ALP was also significant (p<0.05) as compared to controls and dose-dependent (r²=0.963, 0.989 respectively). Activity of ACP was found to increase by 53.12%, 96.87% and 196.87% with treatment of LD, MD and HD of DEP respectively. The increase in serum ALP activity by LD, MD and HD of DEP was 58.33%, 116.66%, 150.00% respectively (Figure 3.36). As compared to vehicle control, DEP treatment in mice (groups 3, 4 and 5) for 30 days caused significant (p<0.05) reduction in protein (LD-18.67%, MD-36.42% and HD-61.77%) and cholesterol (LD-23.69%, MD-35.97% and HD-63.16%) contents dose-dependently (r²=0.992, 0.981 respectively) in serum of mice (Figure 3.37 and 3.39). Oral administration of DEP doses for 30 days significantly (p<0.05) and dose-dependently (r²=0.995) increased serum glucose and creatinine contents. Percent increase in glucose and creatinine contents by LD, MD and HD was 5.40%, 12.25%, 27.58% and 13.54%, 26.86, 36.79% respectively (Figure 3.38 and 3.40).
PART II:

Phytochemical analysis of the *Nigella sativa* seed extract

*Hydro-alcoholic extract:*

As shown in Table 3.16 the *Nigella sativa* seed extract was found to contain considerably large amount of crude phytochemicals as mentioned below:

*Qualitative and Quantitative analysis:*

Table 3.16 represents the results of phytochemical analysis of hydro-alcoholic extract of *Nigella sativa* seed. The extract yield was calculated and presented. The qualitative analysis of *Nigella sativa* seed extract indicated the presence of tannins, saponins, flavonoids and alkaloids. These contents in all the extract was determined quantitatively using standard methods.

**Total phenolic content** (TPC) of the *Nigella sativa* seed extract was estimated using standard method. The concentration of TPC determined in hydro-alcoholic extract of *Nigella sativa* seed was 53.06 mg gallic acid equivalent/gm dry weight calculated using equation that was obtained from standard gallic acid graph (Table 3.16). Table 3.16 shows 7.87 mg quercetin equivalent/gm dry weight of **flavonoid content** in hydro-alcoholic extract of *Nigella sativa* seed which was estimated using quercetin standard graph. Result shown in Table 3.16 revealed that **tannin content** in hydro-alcoholic extract of *Nigella sativa* seed was 4.33 mg rutin equivalent/gm dry weight of extract. **Ascorbic acid content** of hydro-alcoholic extract of *Nigella sativa* seed was 1.21 µg/gm dry weights which was measured by plotting standard ascorbic acid curve (Table 3.16).
Antioxidative potency of Nigella sativa seed extract:

This study was designed to assess the potential of Nigella sativa seed extract to scavenge free radicals using chemical models.

Superoxide radical scavenging assay:

The superoxide radicals generated from PMS/NADH-NBT system were strongly scavenged by various concentrations of Nigella sativa seed extract. The decrease in colour intensity was observed with increasing concentration of extract indicating consumption of the radicals in the reaction mixture (Figure 3.41). Maximum scavenging effect was achieved at 300 μg/mL concentration of the extract. The effect was concentration-dependent for the plant extract ($r^2 = 0.996$). However, the effect was lower than that of standard BHT. Concentration required to scavenge 50% (IC$_{50}$) of the radicals was 247.90 μg/mL for hydro-alcoholic extract of Nigella sativa seed.

Hydroxyl radical scavenging assay:

Hydroxyl radical scavenging capacity of the extract is directly proportional to its antioxidative potency. The percent inhibition of hydroxyl radical increased significantly ($p<0.05$) with increasing concentrations of hydro-alcoholic extract of Nigella sativa seed (Figure 3.42). Results indicated that hydro-alcoholic extract of Nigella sativa seed showed maximum scavenging at 50 μg/mL concentration. IC$_{50}$ value of extract was 36.76 μg/mL. The scavenging effect was concentration dependent ($r^2 = 0.993$), though it was lower than that of BHT.
**Nitrous oxide radical scavenging assay:**

Nitrous oxide radical generated from sodium nitroprusside at physiological pH was significantly (p<0.05) inhibited by hydro-alcoholic *Nigella sativa* seed extract. Percent inhibition was concentration-dependent ($r^2 = 0.993$) and maximum at 250 µg/mL concentration of the extract. IC$_{50}$ value for nitrous oxide scavenging activity was 202.3 µg/mL (Figure 3.43).

**DPPH radical scavenging assay:**

DPPH radical scavenging activity of various concentrations of *Nigella sativa* seed extract was found statistically significant (p<0.05). Decrease in absorbance due to antioxidative effect of *Nigella sativa* seed extract was 250 µg/mL concentration (Figure 3.44). IC$_{50}$ value for the hydro-alcoholic extract of *Nigella sativa* seed was 201.61 µg/mL. Scavenging effect of the extract was concentration-dependent ($r^2 = 0.984$) which was lower than that of standard. It was found that BHT had higher activity than that of *Nigella sativa* seed extract.
PART III

To study the ameliorative effect of Nigella sativa seed extract on toxicity induced by diethyl phthalate

**In vitro study**

**Lipid peroxidation:**

Exposure of liver and kidney homogenates with 40 µg/mL of DEP had resulted in significant (p<0.05) increase in MDA levels-marker of lipid peroxidation as compared to control. Cotreatment of DEP (40 µg/mL) along with various concentrations of hydro-alcoholic Nigella sativa seed extract to the liver and kidney homogenates had significantly (p<0.05) reduced levels of DEP – induced lipid peroxidation. Table 3.17 and 3.18 shows that addition of hydro-alcoholic extract of Nigella sativa seed extract (25, 50, 75, 100, 150 and 200 µg/mL) in 40 µg/mL DEP-treated homogenates had resulted in significant (p<0.05) and concentration – dependent \( r^2 = 0.986 \) and 0.984 respectively) decreases in MDA levels (Figure 3.45 and 3.46).

**Protein content:**

Effect of DEP treatment on protein content was evaluated in vitro condition in liver and kidney homogenates. Results revealed that protein content significantly (p<0.05) decreased with 40 µg/mL DEP treatment (Table 3.17 and 3.18).

Concurrent addition of hydro-alcoholic Nigella sativa seed extract (25, 50, 75, 100, 150 and 200 µg/mL) into DEP (40 µg/mL) – treated liver and kidney homogenates resulted in significantly (p<0.05) increase in protein content (Table 3.17.
Effect was concentration-dependent \( (r^2=0.982, 0.903 \text{ respectively}) \). No significant decrease in protein content was found with hydro-alcoholic *Nigella sativa* seed extract (300 µg/m) treatment only (Figure 3.47 and 3.48).

**In vivo study**

**Clinical observation:**

No treatment related clinical signs were observed in vehicle and *Nigella sativa* seed extract - treated controls (Group 1 and 2). Oral administration of high dose of DEP for 30 days caused dullness and lethargy. Conversely conjoint treatment of DEP and hydro-alcoholic extract of *Nigella sativa* seed completely ameliorated all DEP treatment related clinical changes in mice.

**Body weight:**

Oral administration of high dose of DEP to mice had resulted in significant \( (p<0.05) \) reduction in body weight as compared to untreated and vehicle controls (Table 3.19). Administration of *Nigella sativa* seed extract along with DEP (Groups 4 and 5) caused significant amelioration in body weight, as compared to only DEP-treated group (Group 3). Extent of amelioration was more in high dose of *Nigella sativa* seed extract – treated group as compared to low dose (Table 3.19). Percent change on cotreatment with *Nigella sativa* seed extract is shown in Figure 3.49.

**LIVER:**

**Absolute and relative weights:**

No significant difference was observed between vehicle and antidote controls. High dose of DEP administration significantly increased absolute and relative weight
of liver to 167.86% and 141.30% respectively. Oral administration of *Nigella sativa* seed extract along with high dose of DEP resulted in significant (p<0.05) decrease in absolute and relative weights of liver (Table 3.20; Figure 3.50 and 3.51) as compared to toxin – treated animals. Hepatoprotective index calculated for *Nigella sativa* seed extract for both absolute and relative liver weight were **NS150: 42.10 and NS300: 71.90 and NS150: 51.10 and NS300: 77.00** respectively (Figure 3.50 and 3.51).

**BIOCHEMICAL ANALYSIS**

*Protein, carbohydrate, lipid and nucleic acid contents:*

Table 3.21 shows the protective effect of *Nigella sativa* seed extract on DEP – induced changes in protein, carbohydrate, lipid and nucleic acid contents. Oral administration of 300 mg/kg bw *Nigella sativa* seed extract did not cause any significant change than that of vehicle control. Oral administration of high dose of DEP had resulted in reduction in protein content to 48.80%. The protection denoted by *Nigella sativa* against DEP – induced reduction in protein content was **NS150: 33.40 and NS300: 72.90** as calculated by hepatoprotective index (Figure 3.52). Oral administration of HD of DEP caused significant increase in glycogen content. Glycogen content in the liver of groups 4 and 5 was found to restored back due to protective effect of *Nigella sativa* seed extract (Figure 3.53). Hepatoprotective index showed 33.50 and 61.00 protection denoted by NS150 and NS300 respectively (Table 3.21). Amelioration was more in high dose of *Nigella sativa* extract than that of low dose.

No significant changes in cholesterol and total lipid contents were observed in control groups (Group 1 and 2). Oral administration of high dose of DEP for 30 days
caused, as compared with the vehicle control, significant (p<0.05) increase in cholesterol (Table 3.21) and lipid contents. As compared with high dose of DEP, co-treatment with Nigella sativa seed extract along with high dose DEP caused significant amelioration in total lipid and cholesterol contents in liver of mice. Hepatoprotective index calculated for total lipid and cholesterol contents were NS150: 35.00, NS300: 62.00 and NS150: 50.00, NS300: 79.00 respectively (Table 3.21; Figure 3.54 and 3.55).

Table 3.21 shows protective effect of Nigella sativa seed extract on DEP-induced changes in nucleic acid content in the liver of mice liver. Reduction in DNA and RNA content in high dose of DEP-treated mice was 54.29% and 75.84% respectively. All two doses of Nigella sativa seed extract cotreatment along with DEP increased levels of DNA (Figure 3.56). In the same manner RNA content of the DEP-treated animals were found to increase with cotreatment of two different doses of Nigella sativa seed extract (groups 4 and 5) in mice liver (Figure 3.57). The hepatoprotective index calculated for DNA and RNA contents were NS150: 36.60, NS300: 88.70 and NS150: 32.70, NS300: 83.60 respectively.

**HEPATIC ENZYMATIC ALTERATIONS:**

**Activities of ALT, AST, ACP and ALP in liver:**

No significant changes in the enzyme activities of vehicle control and antidote control groups (Group 1 and 2) were found. Oral administration of high dose of DEP (group 3) for 30 days had significantly increased the activities of hepatic enzymes such as ALT, AST, ACP and ALP which were recovered back by the cotreatment of Nigella sativa seed extract (Table 3.22). All doses of Nigella sativa seed extract
(groups 4 and 5) significantly (p<0.05) mitigated high dose of DEP- induced increase in the activities of ALT and AST. Hepatoprotective index (HPI) for ALT was **NS150: 39.30, NS300: 75.00** (Figure 3.57). Protection against high dose of DEP- induced AST increase by *Nigella sativa* seed extract was **NS150: 55.70, NS300: 83.60** as calculated by HPI (Table 3.21; Figure 3.58).

In the same manner increase in the activities of ALP and ACP were successfully ameliorated by *Nigella sativa* seed extract coadministration with high dose of DEP to mice for 30 days. Amelioration achieved in ALP and ACP activity was **NS150: 52.80, NS300: 78.40** and **NS150: 39.50, NS300: 93.70** respectively as indicated by HPI [Table 3.22 (Figure 3.60 and 3.61)].

**Enzymes involved in energy metabolism:**

No significant changes were observed in the activities of succinic dehydrogenase and adenosine triphosphatase in liver of vehicle control animals (Group 1). Oral administration of 300 mg/kg bw *Nigella sativa* seed extract did not cause any significant change than that of vehicle control (Group 2) (Table 3.28). High dose of DEP treatment reduced activities of hepatic SDH and ATPase to 48.70% and 32.46% respectively as compared to vehicle control. Cotreatment of *Nigella sativa* seed extract along with HD of DEP for 30 days caused significant amelioration in all parameters as compared to HD of DEP alone treated groups [Table 3.23; (Figure 3.62 and 3.63). Percent protection in SDH and ATPase activities were **NS150: 31.00, NS300: 82.00** and **NS150: 27.00, NS300: 58.50** respectively as indicated by HPI.
**Phosphorylase activity:**

Table 3.23 shows effect of DEP treatment on phosphorylase activity in liver and its amelioration by *Nigella sativa* seed extract. No significant changes were observed in phosphorylase activity in between different control groups (groups 1 and 2). Oral administration of high dose of DEP caused significant (p<0.05) decrease in phosphorylase activity in liver. Cotreatment of *Nigella sativa* seed extract along with high dose of DEP for 30 days caused significant amelioration in phosphorylase activity as compared to HD of DEP alone treated groups (Figure 3.64). Percent protection in phosphorylase activity was NS150: 37.00, NS300: 57.00 as indicated by HPI.

**Lipid peroxidation and non-enzymatic and enzymatic antioxidants:**

*Nigella sativa* seed extract is known to possess strong antioxidative potency and was used to combat DEP-induced oxidative stress in this study. In mice liver, no significant changes were found in non-enzymatic (glutathione and ascorbic acid contents) as well as enzymatic (glutathione peroxidase, glutathione reductase, glutathione transferase, catalase and superoxide dismutase activities) antioxidants in between different control groups (Group 1 and 2). There was no significant change in lipid peroxidation in all control groups. Oral administration of high dose of DEP for 30 days caused significant increase in lipid peroxidation in liver of mice. This could be due to significant dose-dependent reduction in non-enzymatic (glutathione and ascorbic acid contents) and enzymatic (glutathione peroxidase, glutathione reductase, glutathione transferase, catalase and superoxide dismutase activities) antioxidants in
DEP alone treated animals as compared to vehicle control (group 1) (Table 3.24 and 3.25). Treatment with *Nigella sativa* seed extract along with HD of DEP shows significant amelioration in DEP-induced lipid peroxidation (Groups 4 and 5). The HPI calculated for *Nigella sativa* seed extract was **NS150: 37.00** and **NS300: 77.10** (Table 3.24; Figure 3.65). Recovery in the content of non-enzymatic antioxidants (glutathione and ascorbic acid contents) was also achieved by *Nigella sativa* seed extract treatment which was **NS150: 45.00, NS300: 88.10** and **NS150: 42.40, NS300: 82.70** (Figure 3.66 and 3.67) as per calculated by HPI respectively.

Table 3.25 shows that enzymatic antioxidants were severely affected by DEP treatment, which were brought back to normal by cotreatment of *Nigella sativa* seed extract. Activities of CAT and SOD were reduced by DEP treatment and found to increase significantly (p<0.05) by various doses of *Nigella sativa* seed extract. Hepatic protection by both doses of *Nigella sativa* seed extract for CAT and SOD activities were **NS150: 44.00, NS300: 83.40** (Figure 3.68) and **NS150: 33.40, NS300: 74.60** (Figure 3.69) respectively. Similarly, the protective effect of *Nigella sativa* seed extract on the activities of GR, GST and GPx were also significant (p<0.05). HPI calculated for GR, GST and GPx were **NS150: 31.50, NS300: 83.50** (Figure 3.70); **NS150: 38.20, NS300: 62.80** (Figure 3.71) and **NS150: 40.90, NS300: 78.50** (Figure 3.72) respectively.

**HISTOPATHOLOGICAL STUDY**

The transverse section of liver of *Nigella sativa* seed extract alone treated mice showed normal hepatocytes and sinusoids and similar morphology as in vehicle control (Plate B and K). Oral administration of high dose of DEP for 30 days caused severe fat deposition, intra – cellular vacuolization, necrosis and loss of histo -
architecture as compared to controls in liver of mice (Group 3) (Plate E). Treatment with two different doses of *Nigella sativa* seed extract (Groups 4 and 5) for 30 days along with HD of DEP caused amelioration in DEP- induced changes as compared to only HD of DEP- treated group (Group 3). Mild reduction in fat deposition and necrosis were achieved with NS150 coadministration (Plate L). Oral administration of *Nigella sativa* seed extract along with high dose of DEP caused almost complete restoration in histo-architecture as indicated in Plate M Amelioration was more in high dose *Nigella sativa* seed extract treated animals than that of low dose.

**KIDNEY:**

**Kidney weight**

Table 3.26 shows effect of DEP on absolute and relative weights of kidney and its amelioration by *Nigella sativa* seed extract. There were no significant effects found in absolute and relative kidney weights in different control groups (Group 1 and 2). Results revealed that high dose of DEP treatment for 30 days caused significant (p<0.05), increase in absolute and relative kidney weights as compared to vehicle control (Group 2). Oral administration of *Nigella sativa* seed extract along with HD of DEP significantly ameliorates DEP – induced changes in absolute and relative kidney weights (Groups 4 and 5) (Figure 3.73 and 3.74). As per shown in Table 3.26 protective percent of *Nigella sativa* seed extract for absolute and relative weights were **NS150: 34.00, NS300: 66.60** (Figure 3.73) and **NS150: 45.00, NS300: 82.00** (Figure 3.74) respectively as per calculated by renoprotective index (RPI).
No significant alterations in protein, total lipid and cholesterol contents were observed in vehicle control group (Group 1). Nigella sativa seed extract (300 mg/kg body weight) only treatment did not cause any significant effect from vehicle control (Group 2). As per shown in Table 3.2, oral administration of high dose of DEP in animals of Group 3 caused significant increase in lipid and cholesterol contents whereas protein content reduced significantly. However, concurrent administration of Nigella sativa seed extract along with high dose of DEP leads to significant amelioration in protein content in Group 4 and 5 animal, when it was compared with high dose of DEP alone treated group (Group 3). RPI calculated for protein content was NS150: 42.00 and NS300: 85.20 (Figure 3.75). Also results revealed significant and dose-dependent reduction in total lipid and cholesterol content in kidney of Nigella sativa seed extract along with DEP - treated animal. Protection denoted by Nigella sativa seed extract in lipid and cholesterol content which was calculated by RPI was NS150: 42.00, NS300: 77.00 and NS150: 26.00, NS300: 63.00 respectively (Table 3.25; Figure 3.76 and 3.77).

In kidney, no significant changes were observed in DNA and RNA contents in all control groups (Group 1 and 2). As compared to animals of vehicle control, high dose of DEP treatment caused (Group 3) significant reduction in DNA and RNA contents (Table 3.27; Figure 3.78 and 3.79). Combined treatment of Nigella sativa seed extract plus high dose of DEP caused significant amelioration in all parameters as compared to high dose of DEP alone treated groups RPI calculated for DNA and RNA contents in kidney were NS150: 41.60, NS300: 77.60 (Figure 3.78) and NS150: 38.00, NS300: 84.20 (Figure 3.79) respectively.

*Enzymes involved in energy metabolism:*
No significant changes were observed in the activities of succinic dehydrogenase, adenosine triphosphatase as well as ACP activities in the kidney of different control groups (Group 1 and 2). Oral administration of high dose of DEP caused significant reduction in SDH and ATPase enzyme activities as compared to control. However, treatment with \textit{Nigella sativa} seed extract along with high dose of DEP caused significant amelioration in these enzyme activities as compared to DEP alone treated group. Percent protection by \textit{Nigella sativa} seed extract upon DEP – induced reduction in SDH and ATPase activities was \textbf{NS150: 30.00, NS300: 62.30} (Figure 3.80) and \textbf{NS150: 49.20, NS300: 75.50} (Figure 3.81) respectively as per calculated by renoprotective index. The oral administration of high dose of DEP for 30 days in mice caused, significant increase in ACP activity in kidney (group 3). Cotreatment of \textit{Nigella sativa} seed extract along with HD DEP caused significant decrease in the activities of ACP. RPI calculated for ACP was \textbf{NS150: 39.50, NS300: 93.70} (Figure 3.82).

\textit{Lipid peroxidation and non-enzymatic and enzymatic antioxidants:}

No significant alterations were found in \textit{Nigella sativa} seed extract (300 mg/kg body weight) treated mice than that of vehicle control in lipid peroxidation, non-enzymatic and enzymatic antioxidants. Oral administration of high dose of DEP for 30 days caused significant increase in lipid peroxidation in kidney of mice (Table 3.29). This could be due to significant dose- dependent reduction in non-enzymatic (glutathione and ascorbic acid contents) and enzymatic (glutathione peroxidase, glutathione reductase, glutathione transferase, catalase and superoxide dismutase activities) antioxidants in kidney of group 3 animals (Table 3.29). Simultaneous
treatment of *Nigella sativa* seed extract and DEP (Group 4 and 5) caused significant amelioration, as compared to DEP alone treated groups, in lipid peroxidation (RPI - NS150: 44.40, NS300: 78.70) (Figure 3.83). Co-treatment of *Nigella sativa* seed extract along with HD DEP (Group 4 and 5) significantly increased the GSH and TAA levels (RPI - NS150: 38.40, NS300: 58.40 (Figure 3.84) and NS150: 47.00, NS300: 79.60 (Figure 3.85) respectively).

DEP treatment caused significant and dose-dependent reduction in CAT and SOD activity which was mitigated by the *Nigella sativa* seed extract (Table 3.30). The effect was significant, (p<0.05) and the RPI calculated was NS150: 35.60, NS300: 63.70 (Figure 3.86) and NS150: 41.10, NS300: 77.70 (Figure 3.87) respectively. In the same fashion, activities of GR, GST and GPx were also restored by the treatment of *Nigella sativa* seed extract along with HD DEP. As per shown in RPI for GR, GST and GPx were NS150: 33.70, NS300: 65.50, NS150: 42.00, NS300: 79.10 and NS150: 20.80, NS300:62.20 (Figure 3.88, 3.89 and 3.90 respectively) (Table 3.30).

**Serum parameters**

Table 3.30 shows the effect of *Nigella sativa* seed extract on DEP-induced changes in serum of mice. No significant difference in activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), acid phosphatase (ACP) as well as total protein, glucose, cholesterol and creatinine contents were observed between different control groups (Groups 1 and 2). Oral administration of DEP significantly increased ALT, AST, ACP, ALP activities as well as creatinine and glucose contents as compared with vehicle control. However, total protein and cholesterol contents were significantly decreased in DEP-treated animals as compared to vehicle control. However, in groups 4 and 5, oral
administration of HD DEP along with *Nigella sativa* seed extract, caused significant mitigation, as compared to DEP- treated mice (Group 3). Amelioration by the *Nigella sativa* ALT, AST, ALP and ACP were **NS150:33.70, NS300:65.50, NS150:42.00, NS300:79.10** and **NS150:20.80, NS300:62.20** (Figure 3.91 to 3.98).

**HISTOPATHOLOGICAL STUDY:**

No significant change was observed in histopathological features of kidney in vehicle control mice (Group1) (Plate G). Oral administration of DEP for 30 days caused distortion of the tubules, increased vacuolization, necrosis, atrophy of the glomerulus and increased space between glomerulus and capsule wall (Groups 3) (Plate J). No apparent histopathological changes were observed in the kidney of *Nigella sativa* seed extract alone treated mice (Plate N). Cotreatment with *Nigella sativa* seed extract (Group 4 and 5) along with DEP (HD) for 30 days showed almost complete recovery in histopathological features; it was almost comparable to vehicle control (Plate O and P).

The results of the Pearson correlation analysis between increase in LPO and depletion of enzymatic and non-enzymatic antioxidants in liver and kidney of mice were shown in Table 3.32 and 3.33 respectively. There is significant negative correlation between elevation in LPO and antioxidants present in the liver and kidney.