CHAPTER V
SUMMARY AND CONCLUSION

Diethyl phthalate is used ubiquitously as solvents and plasticisers worldwide. The present study was undertaken to evaluate diethyl phthalate – induced toxic effects on vital organs of mice (liver and kidney) with sub chronic exposure. Toxic effects of diethyl phthalate on mice liver and kidney homogenates were also studied to get better understanding of the mechanism involved in diethyl phthalate – induced toxicity. 

*Nigella sativa* is an important therapeutic agent in the Indian traditional system of medicine like Unani and Ayurveda. *Nigella sativa* seed extract was used in the study to mitigate diethyl phthalate exerted toxicity in various in vitro and in vivo conditions.

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

*In vitro study*

Diethyl phthalate – induced lipid peroxidation (LPO) was measured in liver and kidney homogenates by quantifying thiobarbituric acid reactive substances (TBARS) *in vitro*. Results revealed that H$_2$O$_2$ – induced LPO was significantly increased as the concentration of diethyl phthalate was increased in liver and kidney homogenates. Maximum increase was found with 40 µg/mL concentration of diethyl phthalate.

Similarly, effect of DEP treatment on protein content was evaluated in *vitro* condition in liver and kidney homogenates. Results revealed that protein content significantly decreased as the concentration of DEP was increased in liver and kidney
homogenates. Maximum reduction in protein content was observed at 40 µg/mL concentration of DEP. This might be due to increased LPO, which may be due to DEP-induced generation of free radicals and oxidative stress.

In vivo study

In vivo study was designed to evaluate hepatic and renal damage caused by administration of diethyl phthalate in mice. Summary of the diethyl phthalate–induced toxicity is as follows:

Diethyl phthalate treatment for 30 days caused dullness and lethargy in treated mice. However, no clinical signs were observed in untreated, as well as vehicle control animals.

Oral administration of all three doses of diethyl phthalate resulted in significant, dose-dependent reduction in body weight the animals as compared to vehicle control. No significant changes in the body weight were noted between different controls (Groups 1 and 2).

LIVER:

In groups 1 and 2 animals no significant differences in absolute and relative liver weight were found. However, oral administration of diethyl phthalate for 30 days caused significant, dose-dependent increase in absolute and relative liver weights (Groups 3, 4 and 5) in mice as compared to vehicle control.

Groups 1 and 2 show no significant alteration in protein, lipid, cholesterol and glycogen contents. When diethyl phthalate was administrated orally, there was significant, dose-dependent decrease in protein content as well as increase in
glycogen, lipid and cholesterol contents in liver of Groups 3, 4 and 5 animals when compared with vehicle control.

Alteration in macromolecule contents of liver such as DNA and RNA was found to be the prominent feature of diethyl phthalate toxaemia. DNA and RNA were found to reduce significantly and dose-dependently with diethyl phthalate oral administration. No significant change was observed between untreated and vehicle control.

No significant changes were observed in the activities of succinic dehydrogenase, adenosine triphosphatase and phosphorylase in liver of different control groups (Group 1 and 2). However, oral administration of DEP caused dose-dependent, significant reductions in the activities of succinic dehydrogenase, adenosine triphosphatase and phosphorylase as compared to vehicle control.

Oxidative stress markers such as non-enzymatic (glutathione and ascorbic acid contents) as well as enzymatic (glutathione peroxidase, glutathione reductase, glutathione transferase, catalase and superoxide dismutase activities) antioxidants along with lipid peroxidation were significantly altered in the liver of DEP-treated mice. Level of lipid peroxidation was increased dose-dependently in DEP-treated animals as compared to control which could be due to the reduction in non-enzymatic and enzymatic antioxidants.

Oral administration of DEP for 30 days caused severe fat deposition, intra-cellular vacuolation, necrosis and loss of histo-architecture as compared to controls in liver of mice. No significant changes were observed in all groups of control animals.
KIDNEY:

There were no significant effects observed in absolute and relative kidney weights in different control groups (Group 1 and 2). Treatment of DEP for 30 days caused significant, dose-dependent increase in absolute and relative kidney weights of mice (Group 3, 4 and 5) as compared to vehicle control.

No significant alterations in protein, lipid, cholesterol contents were observed in different control groups (Group 1 and 2). DEP treatment caused significant decrease in protein content whereas lipid and cholesterol contents were significantly increased (Group 3, 4 and 5) as compared with vehicle control (Group 2).

In kidney, no significant changes in DNA and RNA contents were observed between different control groups (Group 1 and 2). However, DEP treatment caused, significant dose-dependent decrease in DNA and RNA contents (Group 3, 4 and 5), as compared to vehicle control.

There were no significant changes observed in the activities of succinic dehydrogenase and adenosine triphosphatase between different control groups (Group 1 and 2). However, oral administration of DEP caused dose-dependent, significant reductions in the activities of succinic dehydrogenase and adenosine triphosphatase in kidney of mice as compared to the controls.

Results revealed that oral administration of DEP for 30 days caused significant increase in lipid peroxidation in kidney 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 kidney of groups 3, 4 and 5 animals as compared to vehicle control. However, there were no significant alterations found in different control groups in above mentioned parameters.
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). 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). The effect was more prominent in high dose DEP-treated animals than that of low dose.

No significant difference was observed between different controls (Groups 1 and 2). The oral administrations of DEP for 30 days caused significant increase in activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), acid phosphatase (ACP) as well as creatinine and glucose contents in serum of mice. However, total protein, cholesterol contents were significantly reduced in DEP-treated mice. The effect was more prominent in high dose DEP-treated animals than that of low dose.

PART II: Phytochemical analysis of the *Nigella sativa* seed extract

Phytochemicals are the secondary metabolites present in plants in minute concentrations and principally responsible for the medicinal value of the same. Various polyphenol content of hydro-alcoholic extract of *Nigella sativa* seed was quantitatively estimated in the present study. The extract showed presence of phenolics, flavonoids, tannins and ascorbic acid in considerably high amount indicating its good antioxidative potency as phytochemicals are known reductant. Various chemical antioxidant assay systems were used to evaluate free radical
scavenging activity of *Nigella sativa* seed extract. Hydro – alcoholic extract of *Nigella sativa* seed effectively scavenged superoxide, hydroxyl, nitrous oxide and DPPH radicals in *in vitro* condition. Results indicate that hydro – alcoholic extract of *Nigella sativa* seed can effectively reduce the oxidized compounds. Free radical scavenging assay as well as reducing ability showed that hydro- alcoholic extract was potent due to higher amount of phytochemicals present in *Nigella sativa* seed extract.

*Nigella sativa* seed extract was used to study their possible mitigatory effects against diethyl phthalate toxicity in *in vitro* conditions. Results revealed that concurrent addition of *Nigella sativa* seed extract (0-200 µg/mL) along with DEP (50 µg/mL) caused concentration-dependent significant decrease in lipid peroxidation as well as increase in protein content in liver and kidney homogenates. Therefore, *Nigella sativa* seed extract was selected to study its possible amelioration against DEP- induced toxicity in *in vivo* conditions.

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

Efficiency of *Nigella sativa* seed extract in reducing diethyl phthalate exerted toxicity in mice was evaluated using two different doses. Cotreatment of *Nigella sativa* with diethyl phthalate in mice resulted in significant mitigation of diethyl phthalate- induced toxicity in liver and kidney which are as follows:

Cotreatment of *Nigella sativa* seed extract and DEP significantly mitigated DEP- induced clinical symptoms such as lethargy and dullness. No treatment related mortality was observed in any of the experimental groups.

No significant difference in body weight was observed between different control groups (Group 1 and 2). Oral administration of DEP (HD) caused significant
reduction in body weight. However, oral administration of 150 and 300 mg/kg body weight/ day of *Nigella sativa* seed extract along with HD of DEP caused significant mitigation in retardation in body weight, as compared to HD of DEP alone treated animals (Group 3) in a dose – dependent manner.

**LIVER:**

No significant difference in the absolute and relative liver weights was found between different control group animals (Group 1 and 2). As compared to the vehicle control (Group 2), oral administration of DEP (HD) for 30 days caused significant increase in absolute and relative liver weights of mice. *Nigella sativa* seed extract treatment along with HD of DEP significantly reduced absolute and relative weights of liver which was significantly increased with HD of DEP – treatment (Group 3).

Animals of group 1 and 2 show no significant alterations in protein, lipid, cholesterol and glycogen contents. Conversely, as compared to vehicle control (Group 2) oral administration of DEP in animals of group 3 caused significant decrease in protein content, whereas significant rise was observed on glycogen, lipid and cholesterol contents. Concurrent administration of *Nigella sativa* seed extract and HD of DEP leads to significant amelioration in all parameters in liver of Group 4 and 5 animals, when it was compared with groups treated with HD of DEP alone (Group 3).

There were no significant changes in DNA and RNA contents in liver of all control group animals (Group 1 and 2). Significant elevation in DNA and RNA contents were noted in liver of mice treated with DEP (HD) along with *Nigella sativa* seed extract while were reduced in case of diethyl phthalate intoxication.

No significant changes were observed in the activities of succinic dehydrogenase and adenosine triphosphatase in liver of different control group
(Group 1 and 2) animals. 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.

*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 in 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. Treatment with *Nigella sativa* seed extract along with HD of DEP shows significant amelioration in all above parameters (Groups 4 and 5) as compared to HD of DEP alone treated group (Group 3).

The transverse section of liver of vehicle control and antidote control mice showed normal hepatocytes and sinusoids. Oral administration of DEP (HD) for 30 days caused severe fat deposition, intra–cellular vacuolation, necrosis and loss of histo - architecture as compared to controls in liver of mice (Group 3). Treatment with *Nigella sativa* seed extract 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). Amelioration was more in high dose *Nigella sativa* seed extract treated animals than that of low dose.

**KIDNEY:**

There were no significant effects found in absolute and relative kidney weights in different control groups (Group 1 and 2). Results revealed that DEP (HD) treatment for 30 days caused significant, increase in absolute and relative kidney weights as compared to vehicle control (Group 2). Oral administration of *Nigella sativa* seed extract
extract along with HD of DEP significantly ameliorates DEP – induced changes in absolute and relative kidney weights (Groups 4 and 5)

No significant alterations in protein, total lipid and cholesterol contents were observed between different control groups (Group 1 and 2). Oral administration of DEP (HD) 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 and DEP leads to significant amelioration in all parameters in Group 4 and 5, when it was compared with DEP alone treated group (Group 3).

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, DEP treatment caused (Group 3) significant reduction in DNA and RNA contents. Combined treatment of *Nigella sativa* seed extract plus DEP caused significant amelioration in all parameters as compared to DEP alone treated groups.

No significant changes were observed in the activities of succinic dehydrogenase and adenosine triphosphatase in kidney of different control groups (Group 1 and 2). Oral administration of DEP (HD) caused significant reduction, in these enzyme activities as compared to control. However, treatment with *Nigella sativa* seed extract along with DEP caused significant amelioration in all parameters as compared to DEP alone treated group.

Oral administration of DEP (HD) for 30 days caused significant increase in lipid peroxidation in kidney 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 group 3 as compared to vehicle
control. Simultaneous treatment of *Nigella sativa* seed extract and DEP (Group 4 and 5) caused significant amelioration, as compared to DEP alone treated groups, in all parameters. No significant alterations were found in different control groups in above mentioned parameters.

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). 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. No significant changes was observed between vehicle control and antidote control groups (Group 1 and 2).

No significant difference in activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), acid phosphatase (ACP) as well as total protein, creatinine, glucose and cholesterol contents in serum were observed between different control groups (Groups 1 and 2). Oral administration of DEP significantly increased activities of ALT, AST, ACP and ALP 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. These changes were effectively mitigated by the administration of *Nigella sativa* seed extract along with DEP in a dose - dependent manner.

In conclusion, DEP leads to the formation of free radicals and thus it creates oxidative stress which in turn damages the vital organs. DEP also disturbs the energy metabolism by altering the activities of enzymes of mitochondrial function. These
toxic effects of DEP had been mitigated by *Nigella sativa* seed extract because of its antioxidative properties.

**FUTURE PERSPECTIVES:**

Present study was an attempt to establish a correlation between xenobiotic-induced toxicities and its amelioration by medicinal plant. As this thesis provides various biochemical and histopathological evidence for diethyl phthalate – induced toxicity (*in vitro* and *in vivo*) and protective effect of the plant extract against it following are the some of the perspectives for which the study can be extended:

1. Detailed and systematic investigation of mechanism of diethyl phthalate-toxicity in various *in vitro* and *in vivo* models by advanced analytical techniques and provide more scientific research base for the same.

2. Evaluation of diethyl phthalate exposure and toxicity on human beings supported by surveys and clinical trials.

3. Comparative potency evaluation of *Nigella sativa* seed extract on various chemical and animal models.

4. Isolation, purification and characterization of various active components possessing physiological action from *Nigella sativa* seed extract which can be tested further for the management of numerous diseases and disorders.