2.1 INTRODUCTION

Deltamethrin [(1R 3S) [α-cyano (3-phenoxyphenyl) methyl]-3-(2, 2-dibromo-ethenyl)-2, 2-dimethyl-cyclopropanecarboxylate] is a synthetic pyrethroid, worldwide engrossed in agriculture, indoor/outdoor pest management and disease vector control programmes (Yousef et al., 2006). The polluted food and water are the reported important sources where animal and human get exposed to DLM and notably it is observed that DLM readily absorbed via oral route (Barlow et al., 2001). Upon oral administration in rats, DLM is reported to rapidly absorb and excreted in urine (19-47%) and feces (32-55%) within 24 h (Katsuki et al., 1967). The ester cleavage and oxidation at position at the 2’ and 4’ position of the terminal aromatic ring of the alcohol moiety is the main route of their biotransformation. According to reports, the liver was found to accumulate a larger concentration of its metabolites as it is the major site of DLM metabolism (Wang et al., 2013). Moreover, studies have shown that DLM produces dose and time-dependent increment in the cytochrome P450s (CYPs) which involved in its metabolism in rat brain and liver (Godin et al., 2007; Johri et al., 2006). In mammals, various ill effects of DLM have been reported in different tissues and organs, including liver (Abdou and Abdel-Daim, 2014; Xu et al., 2015).

Previously, studies have shown that DLM induced liver hypertrophy enhances the level of kupffer cells, inflammatory cytokines, focal necrosis and causes circulatory disorders (Cengiz and Unlu, 2006). Moreover the hazardous effect of DLM on immunological system has also been suspected. It is a potent neurotoxicant and even at a very low dose, could give rise to potential immunotoxicities In situ (Kote et al., 2006).
this reference, earlier reports have demonstrated that from sub-acute to chronic exposure of pyrethroids like DLM can cause local and systemic immunomodulatory manifestations. These include reduced growth and cellularity of the spleen and thymus, decrease in immunoglobulin levels and contact hypersensitivity reactions with weaken natural killer cell cytotoxicity in rodents (Blaylock et al., 1995; Madsen et al., 1996; Punareewattana et al., 2001). DLM can alter blood parameters, antioxidant defense and amend immune functions. Previously, the members of pyrethroid family had found to incite critical inflection in cellular immune response (Kumar et al., 2016; Kumar et al., 2015; Kumar and Sharma, 2015) but the detail investigation of induced immunomodulatory effect has not been cleared yet. Further, experimental evidences have shown that oxidative stress and inflammatory reactions play a major role in DLM induced toxic manifestations (Amin and Hashem, 2012; Dinu et al., 2010; Jiang et al., 2015). Nonetheless the exact relative contributions of these factors leading to DLM induced chronic liver injury and immune disparities remain in dispute. Hence, in the present we have investigated the effect of DLM on hepatic and immune system of male Wistar rats.

2.2 Material and Methods

2.2.1 Reagents

Decis (deltamethrin, 2.8% E.C.) was received from Bayers Crop Science Ltd (Mumbai). 37% formaldehyde, Concanavalin A (Con A), Lipopolysaccharide (LPS), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), RPMI 1640 medium were purchased from Sigma Chemical Co., St. Louis, MO, USA; Rat cytokines primers, RNA isolation columns and reagents were procured from Invitrogen. The cDNA synthesis
kit was purchased from ABI Applied Biosciences. The rest of the chemicals used in the study were analytical grade of purity and procured locally.

### 2.2.2 Rodent model, maintenance and treatment schedule

Male Wistar rats, (200-250 g body b.wt.) were obtained from animal breeding colony of Indian Institute of Toxicology Research (IITR). Animals were acclimatized for 1 week before the start of the experiment. All the guidelines of Institutional Animal Ethics Committee (ITRC/IAEC/59/2013) were followed for the care and use of laboratory animals. The animals were kept under standard laboratory conditions (temperature 23 ± 2°C, relative humidity 55 ± 5%) and were fed with synthetic pellet basal diet (Ashirwad, Chandigarh, India) and drinking water *ad libitum*.

Briefly, our *In vivo* study was carried out to evaluate the liver and immune damaging potential of DLM. Animals were randomly assigned to different experimental groups (n=5), 8 animals each.

- **Group I**: Vehicle control
- **Group II**: 1/10<sup>th</sup> of LD<sub>50</sub> of DLM: 12.8 mg/kg in corn oil
- **Group III**: 1/25<sup>th</sup> of LD<sub>50</sub> of DLM: 5.12 mg/kg in corn oil
- **Group IV**: 1/50<sup>th</sup> of LD<sub>50</sub> of DLM: 2.56 mg/kg in corn oil

Oral administration of DLM was given to Wistar rats with corn oil (200 µl) for consecutive 7 days. At the end of the experimental period, animals of the different groups were sacrificed and the liver, spleen and thymus were taken accordingly.
2.2.3 Body Weight Gain (%)

The body weight of animals was measured initially on 1st day and on 8th day whereas the organs such as liver, spleen, kidneys and thymus were weighed immediately after sacrifice (on 8th day).

Body weight gain and relative organ weights were calculated accordingly:

\[
\text{Body weight gain (\%)} = \frac{(\text{Final body weights} - \text{initial body weights}) \times 100}{\text{initial body weight}}
\]

And, relative weight of organs = mean of weight of organs of each group/ body weight(s) x 100

2.2.4 Determination of reactive oxygen species (ROS)

For measurement of ROS, firstly tissue samples were homogenized in 1 ml phosphate buffer (0.1 M) containing 5 mM EDTA and protease inhibitor cocktail and centrifuged at 10,000×g for 15 min at 4 ºC and the clear supernatant was used for the analysis (Mitra et al., 2015). A 200 µl of supernatant was incubated with 10 µM DCFH-DA for 15 min in the dark and fluorescence was measured in a plate reader (Synergy H1 Hybrid reader, Biotek) at \( \lambda_{\text{excitation}} = 488 \text{nm} \) and \( \lambda_{\text{emission}} = 520 \text{ nm} \). Data is expressed as a mean of DCF fluorescence/mg protein.

2.2.5 Oxidative stress parameters

Liver tissue samples were rinsed and immediately homogenized (10% w/v) in 0.1 M phosphate buffer and centrifuged at 2500×g for 15 min. The resulting supernatants were used for various biochemical analysis such as lipid peroxidation (Varshney and Kale, 1990) Glutathione-S-transferase (GST) activity (Habig et al., 1974), antioxidant activities of catalase (Sinha, 1972) and superoxide dismutases (SODs) (Kakkar et al., 1984) as described elsewhere.
2.2.6 Histopathological assessment

For histological analysis, a portion of liver tissue from animals of different groups was cut randomly, washed and stored in 10% buffered formalin solution. Tissue was embedded in paraffin block and cut in 3-5 nm sections using motorized rotary microtome (RM 2155, Leica, Germany). Slides were stained with hematoxylin and eosin (H&E) and examined and photographed under compound light microscope using Leica DCF-280 camera controlled with software (Leica application suite Ver.3.5.0, Leica, Germany) to observe histopathological changes.

2.2.7 Liver functional test and hematological analysis

Liver function tests by measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT) were assayed. Coagulated blood was collected and centrifuged at 2000× g for 30 min at 4 ºC and clear serum was carefully taken out in a fresh tube. Hematological parameters were estimated by automated hematology cell counter (Model-ms4e). Differential leukocyte count was analyzed as per standard method on the same day on automated Biochemical Clinical Auto Analyzer (Chem Well, USA) using kits from SPINREACT (Spain).

2.2.8 Western blotting

Tissue samples were homogenized with ice-cold PBS, washed with RBC lysis buffer (0.15 M NH₄Cl, 1 M NaHCO₃, 0.1 mM EDTA, pH 7.4) to remove RBCs. Using homogenization buffer (0.25 M sucrose, 10 mM NaCl, 5 mM MgCl₂, 50 mM Tris, 0.2% Triton X-100, pH 7.5, 10 Mm NaF, 2 mM Na₃VO₄,10 µg/ml aprotinin, and 10 µg/ml pepstatin A) tissue lysate was prepared and centrifuged at 12,000×g for 20 min at 4°C. Protein estimation was done by the Bradford assay using bovine serum albumin as standard. Further,
immunoblotting of samples was performed as described earlier using the protocol of Arora et al. (Arora et al., 2013)

2.2.9 Splenocyte culture

Spleens were dissected out, washed with incomplete DMEM and minced well in incomplete DMEM. Cell suspension was treated with erythrocytes lysis buffer (0.15 M NH₄Cl, 1 mM NaHCO₃, 0.1 mM EDTA, pH 7.4) to remove erythrocytes. The cells were subsequently washed three times with incomplete medium. Cells were centrifuged (300 x g) for 5 min at 4 °C and re-suspended in DMEM (containing 10% fetal bovine serum, 1 mM sodium pyruvate, 100 U/ml of penicillin, 100 µg/ml of streptomycin, 25 mM HEPES, 2 mM l-glutamine, 25 mM dextrose and 50 µM 2-mercaptoethanol) for single cell suspension.

2.2.10 Lymphoproliferation assay

The cells were cultured (2×10⁶ cells/ml) in 96 well plates and 8×10⁶ /ml in 48 well plates and incubated overnight for acclimatization. T and B cells in splenocytes were induced with Concanavalin A (Con A) and Lipopolysaccharide (LPS) to proliferate, respectively. Cultured splenocytes were treated with Con A (5 µg/ml) or LPS (10 µg/ml) and incubated at 37 °C for 72 h. Alamar Blue Assay was used to determine the fold proliferation in splenocyte population. Alamar Blue reagent was added to the cultures 4 h prior to the end point. After completion of incubation period, absorbance (OD) of each well was recorded at 600 nm and 570 nm using a 96-well plate reader (BioTek, USA). For BrdU incorporation, 3 µM BrdU was added in culture 18 h prior to the end point. After completion of incubation period, cells were washed with PBS and labeled with Anti-BrdU (Alexaflour 488) for next 15 min. Thereafter cells were acquired by flow cytometry.
2.2.11 Statistical analysis

Data are expressed as mean ± S.D. of at least three rats in the group. Statistical evaluation was performed using one way analysis of variance (ANOVA) followed by Dunnet post hoc tests (GraphPad Prism 5). The level of statistical significance was set at $P<0.05$.

2.3 Results

Animals were placed randomly in four groups (I, II, III and IV) and treated as per schedule described in materials and methods section. During the course of experiment, high mortality rate (approx. 50%) was observed in Group IV (1/$10^{th}$ of LD$_{50}$; 12.8 mg/kg) animals. Therefore, group IV animals were not included in the study. Rests of the groups (I, II, III) were found normal with no signs of aggressive behavior.

2.3.1 Effects of DLM on body weight and relative organ weights

At the end of treatment period, the body weight of rats were significantly ($p< 0.05$) reduced in DLM treated groups as compared to vehicle controls. Statistically significant differences in relative weights of liver, spleen and thymus were noted in all DLM-treated groups at day 7 following exposure (Table 2.1).
Table 2.1: Effect of DLM on body growth and organ weights of Wistar rats

<table>
<thead>
<tr>
<th>Parameters / treatments</th>
<th>Body weight gain (g)</th>
<th>Relative weight of kidney</th>
<th>Relative weight of liver</th>
<th>Relative weight of spleen</th>
<th>Relative weight of thymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>36.67 ± 4.94</td>
<td>0.3857 ± 0.08</td>
<td>4.04 ± 0.56</td>
<td>0.5121±0.05</td>
<td>0.0903±0.08</td>
</tr>
<tr>
<td>1/50\textsuperscript{th} LD50 DLM</td>
<td>24.17 ± 3.27\textsuperscript{*}</td>
<td>0.4251 ± 0.08</td>
<td>3.7596±1.03\textsuperscript{*}</td>
<td>0.4470±0.04</td>
<td>0.0686±0.09\textsuperscript{***}</td>
</tr>
<tr>
<td>1/25\textsuperscript{th} LD50 DLM</td>
<td>20 ± 0.83\textsuperscript{**}</td>
<td>0.4754 ± 0.06\textsuperscript{***}</td>
<td>3.24586±0.15\textsuperscript{***}</td>
<td>0.3485±0.08\textsuperscript{**}</td>
<td>0.0551±0.04\textsuperscript{***}</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD for three rats. Where *p<0.05, **p<0.01, ***p<0.001 vs. control

2.3.2 Effect of DLM on liver function and hematology

DLM treated animals showed a marked reduction in weight of liver tissue. Therefore, next we investigated the effect of DLM on liver. First of all we studied the liver function markers in untreated and DLM treated after 7 days of exposure. Our biochemical analysis of serum from DLM-treated animals showed significant differences in the activity of liver function markers. A significant increase in the activities of AST, ALT and ALP in DLM treated groups suggested the damage to liver function (Table 2.2). Further, we also assayed hematology parameters under similar treatment conditions. A statistically significant decrease in WBC, RBC, PLCR (indicating abnormal plate count), lymphocytes and monocytes and an increase in neutrophil count was noted in DLM treated animals (Table 2.3). No other significant differences were noted in any of the remaining clinical pathology parameters examined such as hemoglobin, Mean corpuscular volume (MCV).
### Table 2.2: Effect of DLM on liver functional markers

<table>
<thead>
<tr>
<th>Parameters/treatments</th>
<th>Control</th>
<th>1/50&lt;sup&gt;th&lt;/sup&gt; LD50 DLM</th>
<th>1/25&lt;sup&gt;th&lt;/sup&gt; LD50 DLM</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (GOT) (mg/dl)</td>
<td>139 ± 5</td>
<td>197.5 ± 8.5**</td>
<td>222 ± 4***</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>595 ± 10</td>
<td>890 ± 2**</td>
<td>912.5 ± 6.5***</td>
</tr>
<tr>
<td>ALT (GPT) (U/l)</td>
<td>52 ± 3</td>
<td>78.5 ± 7.5</td>
<td>100.5 ± 7.5**</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD for three rats. Where *p<0.05, **p<0.01, ***p<0.001 vs. untreated control

### Table 2.3: Effect of DLM on hematological parameters

<table>
<thead>
<tr>
<th>Parameters/treatments</th>
<th>Control</th>
<th>1/50&lt;sup&gt;th&lt;/sup&gt; LD50 DLM</th>
<th>1/25&lt;sup&gt;th&lt;/sup&gt; LD50 DLM</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>23.89 ± 2.3</td>
<td>14.495 ± 1.6**</td>
<td>12.94 ± 0.72***</td>
</tr>
<tr>
<td>RBC</td>
<td>8.275 ± 6.3</td>
<td>8.16 ± 4.6</td>
<td>7.215 ± 0.72</td>
</tr>
<tr>
<td>PLCR</td>
<td>8.05 ± 0.49</td>
<td>6.05 ± 1.9</td>
<td>5.45 ± 0.09*</td>
</tr>
<tr>
<td>NEUTROPHIL</td>
<td>1.425 ± 0.57</td>
<td>2.275 ± 0.02</td>
<td>3.465 ± 0.07*</td>
</tr>
<tr>
<td>LYMPHOCYTE</td>
<td>20.135 ± 5.4</td>
<td>15.36 ± 4.8*</td>
<td>12.46 ± 0.72**</td>
</tr>
<tr>
<td>MONOCYTES</td>
<td>1.425 ± 0.296</td>
<td>0.45 ± 0.09</td>
<td>0.375 ± 0.77**</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD for three rats. Where *p<0.05, **p<0.01, ***p<0.001 vs. untreated control
2.3.3. Effect of DLM on liver histology

Histological examination of liver sections from rats exposed to DLM for 7 consecutive days showed severe abnormalities as compared to control rats. Liver section of unexposed rats exhibited normal polyhedral hepatocytes and the boundaries of sinusoids exhibited single layer of fenestrated endothelial cells with kupffer cells (Figure 2.1). In $1/50^{th}$ LD50 animals, hepatocytes were characterized by the appearance of inflammatory leukocyte infiltrations, activated kupffer cells, dilation in blood sinusoids, dilated congestion, extensive cytoplasmic vacuolization and foci of necrosis. Interestingly the liver section of $1/25^{th}$ LD50 rats showed utmost damage. In fact, at high dose of DLM, infiltration of inflammatory cell aggregate, dilated congested sinusoidal spaces, fatty and degenerative changes, stromal edema, areas of hemorrhages and fibrosis from one inflamed portal tract to another, bridging necrosis/fibrosis were noted.

**Figure 2.1**: Effect of DLM on histopathology of liver tissue. Representative images (40 X) showing histological features (H&E staining) in rat liver following DLM exposure. Images in insets are showing morphology of rat liver at 10 X objective.
2.3.4 DLM induces oxidative stress and antioxidant defense

To determine the effect of DLM on oxidative stress, liver tissues from treated animals were subjected to reactive oxygen species (ROS) analyses on 8th day following exposure. A significant increase in the level of ROS was noted in liver tissue of DLM treated rats as measured by DCFDA fluorescence. Similarly the dose-dependent increment in the LPO and GST in DLM treated groups further supported the load of free radicals in liver. In line to the ROS generation, oxidative modification (LPO) was lesser as compare to DLM groups. Further, for evaluating the effect of DLM on antioxidant defense system (Table 2.4), we assess the activity of antioxidant enzymes SOD (a scavenger of superoxide anions) and CAT (a scavenger of H$_2$O$_2$) enzymes. At higher doses, a significant decrease in enzyme activity of both SOD and CAT was noted. However, at low dose the increase in activities of these enzymes indicated the preconditioning effect. Overall, data suggests that DLM induces the oxidative damage in rodents.
Table 2.4: Effect of DLM on oxidative stress and antioxidant defense

<table>
<thead>
<tr>
<th>Parameters /treatments</th>
<th>Control</th>
<th>1/50&lt;sup&gt;th&lt;/sup&gt; LD50 DLM</th>
<th>1/25&lt;sup&gt;th&lt;/sup&gt; LD50 DLM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT activity (µmoles/min/mg protein)</td>
<td>50.98± 0.12</td>
<td>65.76± 0.50***</td>
<td>24.84± 0.28***</td>
</tr>
<tr>
<td>SOD (IU/mg protein)</td>
<td>8.33± 0.03</td>
<td>10.03±0.08***</td>
<td>4.70± 0.05***</td>
</tr>
<tr>
<td>GST (nm/min/mg protein)</td>
<td>79± 0.03</td>
<td>61± 0.046</td>
<td>52± 0.05**</td>
</tr>
<tr>
<td>LPO (nm TBARS/mg protein)</td>
<td>2.37 ± 0.14</td>
<td>5.9 ± 0.06***</td>
<td>9.82 ± 0.16***</td>
</tr>
<tr>
<td>ROS (OD/mg protein)</td>
<td>0.086 ± 0.02</td>
<td>0.12 ± 0.02***</td>
<td>0.19 ± 0.03***</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD for three rats. Where *p<0.05, **p<0.01, ***p<0.001 vs. untreated control.

2.3.5 DLM exposure induced inflammation in rat liver

DLM-induced histopathological changes in liver tissue clearly verified that DLM stimulates inflammatory response. We also investigated the effect of DLM on liver inflammation. For this, Western blot analysis of various inflammatory markers such as TNF-α, iNOS, IL-6, NF-κB, COX-2, and ICAM in liver tissue lysates was performed. Following treatment, our results showed that protein expression of COX-2, TNF-α, IL-6, and ICAM-1 was up-regulated (a 2-3-fold change) in a dose dependent manner in DLM treated groups (Figure 2.2). However, the protein expression of iNOS and NF-κB remained same at both the doses of DLM.
2.3.6 Immunosuppressive potential of DLM

For the assessment of effect of DLM on immune system we assessed the immune functional response of DLM treated spleen cells against lipopolysaccharide (LPS) and concanavalin A (Con A) challenge. Proliferative responses of spleen were measured after stimulation with mitogens Con A for T cells, and LPS for B cells. The dose dependent decrease in the percentage proliferation population and proliferation index (Figure 2.3A and B) of splenocytes in DLM treated groups has been observed that indicated the suppression of both T cells and B cells population. Overall, our data suggested that DLM also possess immunosuppressant activity.
Figure 2.3: Effect of DLM on functional response of immune cells. (A) Bars (n=3) indicated the percentage population of BrdU positive cells. (B) Bars (n=3) Showing the proliferation index of individual groups calculated with or without treatment of Con A and LPS. Where \( ^a p<0.05 \) vs. untreated control and \( ^b p<0.05 \) vs. stimulant control.

2.4 Discussion

Health apprehensions towards pesticides have tended to focus on their potential by which they pose serious risk to humans. Previously, studies have shown that a variety of pesticides (organochlorine, organophosphate, carbamate etc.) and mixture of chemicals even at low doses on regular exposure affects not only liver metabolic/defense mechanism also suppress the immune system of mammals (Gowdra et al., 2014; Lasram et al., 2014; Rodriguez-Alcala et al., 2015; Toledo-Ibarra et al., 2016). In the present study, we evaluated the effect of DLM dosages on rodent liver and immune system. It is comprehended that stimulation of oxidative stress, inflammation, and immunity imbalance...
generally perturb the physiology and affects the non targets following exposure to xenobiotic compounds including pesticides. Here, in this study, we observed that DLM even at doses 2.56 mg/Kg and 5.12 mg/Kg can generate the significant changes in rodents that could be the responsible factors for the toxic impact of DLM on mammals. Off note, the dose of 12.8 mg/Kg was noted to be quite toxic which showed 50% mortality within three days of treatment schedule. We found that DLM effected the growth of rats in a dose dependent manner on consecutive 7 days oral exposure. Our data is in line with those earlier reports which advocated that DLM via oral route causes severe toxic effects (Ismail and Mohamed, 2013; Yavuz et al., 2015). At the end of the experimental period, the significant reduction in body weight gain as well as in liver, spleen and thymus relative organ weight was also noted. The reduced weight gain is ascribed to the effect of insecticides on gastrointestinal tract resulting in decreased appetite and assimilation of nutrients from gut (Sankar et al., 2012).

We also noted a decrease in spleen and thymus weight that may be indicative deleterious effect of DLM on immune system (Chauhan et al., 2007; Enan et al., 1996; Rehman et al., 2011). Moreover the observed increase in the relative kidney weight in DLM treated animals could be due to congestion of vessels and lymphocytic infiltration during exposure of toxins. Transaminases (AST and ALT) are important enzymes in the biological process as they have important role in amino acids catabolism and biosynthesis. These enzymes were secreted to blood during hepatocellular injury. We have also observed an increase in the level of transaminases (AST, ALT) and AKP in DLM treated animals (Table 2.3). Further the dose dependent decrease in A/G ratio and increase in total proteins correspond to the chronic inflammatory and hepatotoxic effect of DLM.
Previously, studies have demonstrated that generation of ROS is one of the inherent events in pyrethrins metabolism. We also found that DLM could enhance ROS generation in rats. Being a lipophilic substance, DLM like other pyrethrins is known to interact with cellular plasma membrane and by stealing electrons from lipids it causes irreversible damage to them. The dose-dependent increment in LPO further corroborated the DLM induced oxidative stress. Cellular enzymatic as well as non enzymatic antioxidant defense plays a powerful role in combating excessive production of ROS. Oxidative stress builds up when the generation of ROS exceeds the cellular antioxidant defense. Hence we speculated that DLM induced ROS generation could arise due to impaired antioxidant defense. In fact, decreased SOD and CAT activity (antioxidant enzymes) clearly demonstrated that DLM impaired liver enzymatic defense leading to ROS accumulation. Interesting to note that, at low dose of DLM we observed a significant increase in the activity of these enzymes and probably that could initiate a preconditioning effect. Therefore, in spite of DLM toxicity at low dose (1/50th LD50 of DLM), no severe alterations in cellular enzymatic defense were noted as evident from our ROS and enzymatic defense observations in DLM treated animals. Further, a dose dependent decrease in GST (a phase II detoxification) also signified the perturbation of body defense on DLM exposure. Overt ROS generations signified that DLM could affect liver via oxidative damage. In fact enhanced LPO formation and altered redox homeostasis demonstrated the deleterious effect of DLM on liver histology. This apprehension was strengthened by our observations whereas prominent fatty and degenerative changes with inflamed portal tract, infiltration of inflammatory cells and the presence of necrotic foci with areas of bridging fibrosis, edema and hemorrhages were present in DLM treated animals. As evident from histological
analysis that DLM induced toxicity is accompanied with increased inflammation, therefore, we also assessed the level of certain inflammatory markers in treated groups. The dose dependent increase of inflammatory markers such as IL-6, TNF-α, COX-2, iNOS, and ICAM-1 further strengthened that DLM could mount a strong inflammatory response in animals following exposure.

Evidences suggest that any chemically-induced perturbation of the host's hepatic and immune system may compromise its protective capacity and lead to adverse health consequences for its host (Enan et al., 1996). The transfer of reactive metabolites from liver to lymphoid organs results in modulation of the innate and humoral immune response which would attribute to the infection state. Previously it has been reported that DLM enhances the leukocytes count on LPS stimulation in immunogenic cells (Pimpao et al., 2008). However, in the present study we found the decreased T cell proliferation response against Con A and decreased B cell proliferation response against LPS stimulation in Ex vivo culture of splenocytes of DLM treated group (Figure 2.3) which showed the inhibitory effect of DLM on immune cells proliferation.

2.5 Conclusion
The present study demonstrated that the DLM-induced oxidative stress and accompanied inflammation were the key entities that played important role in DLM elicited toxicity. DLM even at acute exposure has the potential to incite hepatotoxicity that would lead to degenerative changes and causes liver fibrosis in rodents. Moreover, upon exposure DLM also stimulated immune alteration by mounting an immunosuppressive response leading to the deprivation of B and T cells. However, further delineation of early responsive signatures of DLM elicited pathogenesis requires more comprehensive evaluation.