Chapter V

Liver Glycogen, LPO & GSH
Liver glycogen

The data presented in Table 5.1 and Fig. 5.1 indicates the liver glycogen levels of 7 experimental groups. In group IV (CCL₄ treated alone) showed significant (p<0.05) decreased levels of glycogen with normal control rats (group I) whereas, group II (Cur. Control) and group III (Cur+Vit.E control) not showed significant difference over normal control. Group V (CCL₄+Cur), VI (CCL₄+Cur. with Vit. E) and VII (CCL₄+Sylamarin) showed the significantly (p<0.05) increased levels over the group IV, whereas, group V, VI and VII showed slightly significant decreased levels of glycogen with normal control group. Group VI showed a slightly significant increased level of glycogen over group V and almost nearer values with group VII (sylamarin treated group).

Glycogen is the main source of energy in liver and is also utilized to maintain blood glucose levels. In the present study rats intoxicated with 8 weeks of CCL₄ discontinuous treatment showed significant depleted levels of liver glycogen content. In supporting of this Muriel P. et al, 2005, has reported that chronic administration of CCL₄ decreased importantly and significantly the liver glycogen content. Kudryavtseva M.V. et al, 2001 reported that changes in the level of glycogen and its fractions in the liver tissue might have resulted from corresponding changes of the hepatic enzymes responsible for synthesis or degradation of glycogen. They measured activities of three enzymes of glycogen metabolism in the liver biopsies of the experimental animals. However, the only changes revealed in the experiments were a decrease of the Glucose-6-phosphatase activity in cirrhosis and its gradual increase after the end of the effect of the hepatotoxic agent.

In the present study treatments of curcumin, curcumin along with vitamin E for 8 weeks not showed significant variations over the untreated control with reference to the liver glycogen levels. CCL₄ along with curcumin treatment significantly (p<0.05) recovered the liver glycogen contents compared to CCL₄ treated ones. Curcumin along with vitamin E significantly recovered (p<0.05) liver glycogen than curcumin treated as well as CCL₄ treated group and not significant difference with CCL₄ with sylamarin treated group. These trends confirm that CCL₄ depleted liver glycogen content were normalised by curcumin and vitamin E. In support of our results, Reyes-Gordillo K. et al., 2008, has reported that bile duct ligation (BDL)
significantly decreased liver glycogen content, but curcumin preserved almost completely this parameter. One explanation is curcumin preserves glycogen by its antifibrotic mechanism because fibrosis disrupts the blood flow to the liver not allowing absorption of nutrients. Moreover, necrosis was induced by BDL and this effect was partially prevented by curcumin, and as necrotic cells do not store glycogen, the antinecrotic effect of curcumin may, in part, explain the preservation of glycogen.

Curcumin is at least 10 times more active as an antioxidant than vitamin E (Kowluru A.R and M. Kanwar 2007). Fu Y et al., 2008 reported that they assumed that overdose of curcumin might be used in their experiments compared with curcumin at 100 mg/kg in some other reports (Sreepriya and Bali, 2006). An even lower dose of curcumin at 40 mg/kg seemed to be effective in the treatment of mice with cystic fibrosis (Egan et al., 2004). So in the present study we have chosen curcumin along with vitamin E for the treatment of hepatic fibrosis induced by chronic CCl₄ administration. Results suggested that curcumin along with vitamin E recovered glycogen content almost nearer to sylamarin treated group and significantly different from curcumin treated and CCl₄ treated ones.
Table: 5.1 Anti-hepatotoxic effect of curcumin and curcumin along with vitamin E on liver damaged by chronic CCl₄ treated rats on liver
Glycogen content, Glutathione content and lipid peroxidation expressed as TBARS formation

<table>
<thead>
<tr>
<th>S.No</th>
<th>Parameter</th>
<th>Group I (Vehicle control)</th>
<th>Group2 Curumin control</th>
<th>Group3 Cur+ Vit E control</th>
<th>Group4 (CCl₄)</th>
<th>Group5 (CCl₄+ Cur)</th>
<th>Group6 CCl₄+ Cur, Vit E</th>
<th>Group7 CCl₄ + Sylamarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Glycogen (mg/gm)</td>
<td>Mean</td>
<td>22.23 ±0.5376</td>
<td>22.36 ±0.3431</td>
<td>22.86 ±0.13</td>
<td>13.53 ±0.4</td>
<td>18.75 ±0.21</td>
<td>20.6 ±0.33</td>
</tr>
<tr>
<td>2.</td>
<td>Lipid peroxidation (nmol/mg)</td>
<td>Mean</td>
<td>1.8167 ±0.0359</td>
<td>1.8485 ±0.0381</td>
<td>1.8133 ±0.0547</td>
<td>17.7493 ±0.4013</td>
<td>3.7033 ±0.3671</td>
<td>2.5765 ±0.2</td>
</tr>
<tr>
<td>3.</td>
<td>GSH (µg/mg)</td>
<td>Mean</td>
<td>22.056 ±0.29</td>
<td>22.03 ±0.159</td>
<td>21.85 ±0.136</td>
<td>10.91 ±0.36</td>
<td>16.83 ±0.295</td>
<td>19.373 ±0.255</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M
Values with different superscripts with in the column are significantly different at P<0.05 (Duncan’s Multiple Range Test)
Figure: 5.1 Effect of curcumin and curcumin along with vitamin E on liver glycogen levels of rats treated with CCI₄. Group I- vehicle control, Group II- curcumin control, Group III- curcumin + vitamin E control, Group IV- CCI₄ treated, Group V- CCI₄ + curcumin treated, Group VI- CCI₄ + curcumin along with vitamin E, Group VII- CCI₄ + Sylamarin (reference drug) treated.
Lipid peroxidation

LPO in liver tissue was studied in 7 experimental groups and data presented in Table 5.1 and Fig. 5.2. Normal rats treated with curcumin (group II) and curcumin along with vitamin E (group III) showed no significant increase in the LPO in liver of N (normal) rats. Group IV (CCI treated group) showed significantly (p<0.05) increased levels of MDA with control group, whereas, group V, VI and VII showed the significantly (p<0.05) decreased levels. Group VI (curcumin with vitamin E) showed a significant decreased level of MDA over group V (curcumin treated ones) and not significantly different with group VII (sylamarin treated group).

In this study rats intoxicated with 8 weeks of CCl4 discontinuous treatment significantly showed increased MDA of the liver tissue when compare to the N control rats. CCl4-induced lipid peroxidation is highly dependent on its bioactivation to the trichloromethyl radical and trichloromethyl peroxy radical (Recknagel RO, 1983; Recknagel RO, 1966; Durk H, Frank H, 1984). It is well known that CCl4 is activated by the cytochrome P450 system. The initial metabolite is the trichloromethyl free radical, which is believed to initiate the biochemical events that ultimately culminate in liver cell necrosis (Mansuy D et al., 1980; Pohl L et al., 1984). The trichloromethyl radical can form covalent adduct with lipids and proteins, interact with O2 to form a trichloromethyl peroxy radical or abstract hydrogen atoms to form chloroform (Pohl L et al., 1983). Other products include conjugated dienes, lipid hydroperoxides, malonaldehyde-like substances, and other short-chain hydrocarbons. In response to hepatocellular injury initiated by the biotransformation of CCl4 to reactive radicals, "activated" Kupffer cells in liver respond by releasing increased amounts of active oxygen species and other bioactive agents (Tirkey N et al., 2005).

Lipid peroxidation is an important parameter of oxidative stress. The increase in liver MDA levels induced by CCl4 suggests enhanced lipid peroxidation, leading to hepatic tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals (Madhusudanarao Vuda et al., 2011). Free radical scavenging is one of the major antioxidation mechanisms inhibiting the chain reaction of lipid peroxidation.
In the present study treatments of curcumin, curcumin along with vitamin E for 8 weeks not showed (table: 5.1, fig. 5.2) variations significantly over the untreated control with reference to the liver lipid peroxide levels. CCl₄ along with curcumin treatment attenuated oxidative stress demonstrated by the reduction in the levels of lipid hydroperoxide in the CCl₄ rat model. Lipid hydroperoxide levels were significantly (p<0.05) diminished compared to CCl₄ treated ones. Curcumin along with vitamin E significantly suppressed (p<0.05) liver hydroperoxide levels than curcumin treated as well as CCl₄ treated group and not significant different from CCl₄ with sylamarin treated group. These trends confirm that CCl₄ elevated liver lipid peroxide levels were normalised by treatment with curcumin and vitamin E. These observations are supported by other studies. Treatment with 100 mg/kg curcumin prevented the drop in the content of hepatic GSH, diminished lipid peroxidation, and minimized hepatocarcinogenesis in rats (Sreepriya and Bali, 2006).

Glutathione (GSH)

The data presented in Table. 5.1 and Fig. 5.3 indicates the liver glutathione (GSH) levels of 7 experimental groups. In group IV (CCl₄ treated alone) showed significantly (p<0.05) decreased levels of glutathione with normal control rats (group I) whereas, group II (Cur. Control) and group III (Cur+Vit.E control) not showed significantly different over normal control. Group V (CCl₄+Cur), VI (CCl₄+Cur. with Vit. E) and VII (CCl₄+Sylamarin) showed the significantly (p<0.05) increased levels over the group IV whereas, with normal control group V, VI and VII showed slightly significant decreased levels of glutathione. Group VI showed a slightly significant increased level of glutathione over group V and almost nearer values with group VII (sylamarin treated group).

GSH is an important non-enzymic antioxidant which promotes the detoxification of several toxic metabolites. The depletion of GSH due to whatever reasons ultimately promotes generation of ROS and oxidative stress with a cascade of effects thereby affecting functional as well as structural integrity of cell and organelle membrane (Karthikeyan J and P. Rani, 2003; Sreepriya and Bali, 2006). CCl₄ induced lipid peroxidation results in the generation of ROS like the superoxide anion O₂', H₂O₂ and hydroxyl radical, OH'. ROS affect the antioxidant defense mechanisms,
decrease the intracellular concentrations of reduced glutathione and reduces the activity of SOD (Srilaxmi P et al., 2010).

In this study rats intoxicated with 8 weeks of CCl₄ discontinuous treatment showed (table. 5.1 and fig. 5.3) significantly decreased the levels of liver glutathione. Treatments of curcumin, curcumin along with vitamin E for 8 weeks not showed variations significantly over the untreated control with reference to the liver glutathione levels. CCl₄ along with curcumin treatment significantly (p<0.05) recovered the liver glutathione levels compared to CCl₄ treated ones. Curcumin along with vitamin E significantly recovered (table. 5 and fig. 16) (p<0.05) liver glutathione than curcumin treated as well as CCl₄ treated group and not significant different with CCl₄ with sylamarin treated group. These trends confirm that CCl₄ depleted liver glutathione content were normalised by curcumin and vitamin E. In support of this Zheng and Chen, 2007 reported that curcumin elevated the level of cellular GSH and induced de novo synthesis of GSH in HSC by stimulating the activity and gene expression of GCL (Glutamate-cystein ligase), a key rate-limiting enzyme in GSH synthesis. It was further demonstrated that de novo synthesis of GSH was a prerequisite for curcumin to inhibit HSC activation (Zheng and Chen, 2007). In this study, they hypothesized that curcumin might protect the liver against CCl₄-induced injury and fibrosis by attenuating oxidative stress. Results in this report indicated that oral administration of curcumin not only increased the level of total hepatic GSH but also significantly improve the ratio of GSH/GSSG in the liver. Parola and Robino, 2001 reported that lipid peroxidation and necrosis are significantly suppressed in the liver of animals supplemented with antioxidants such as flavonoid, silymarin, or vitamin E.
Figure: 5.2 Effect of curcumin and curcumin along with vitamin E on liver peroxidation (MDA) of rats treated with CCl₄.

Group I- vehicle control, Group II- curcumin control, Group III- curcumin + vitamin E control, Group IV- CCl₄ treated, Group V- CCl₄ + curcumin treated, Group VI- CCl₄ + curcumin along with vitamin E, Group VII- CCl₄ + Sylamarin (reference drug) treated.
Figure: 5.3 Effect of curcumin and curcumin along with vitamin E on liver glutathione levels of rats treated with CCl₄.

Group I- vehicle control, Group II- curcumin control, Group III- curcumin + vitamin E control, Group IV- CCl₄ treated, Group V- CCl₄ + curcumin treated, Group VI- CCl₄ + curcumin along with vitamin E, Group VII- CCl₄ + Sylamarin (reference drug) treated.