6.6. HISTOPATHOLOGICAL STUDIES

6.6.1 Average wet liver weight:

After removal of blood, the abdomen of each animal was cut opened and the liver sample was removed, weighed and the ratio of wet weight was calculated.

The results were tabulated in tables 39-42 and represented in bar diagram 40-42.

The results were tabulated in tables: 40-47 and figure no.40-47

**Table No 36: Results of AC and LC on average liver weight of treated animals against Paracetamol**

<table>
<thead>
<tr>
<th>Design of treatment</th>
<th>Liver weight (g) /100g of body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Vehicle 1ml/kg/day, p.o)</td>
<td>2.51 ± 0.42</td>
</tr>
<tr>
<td>Paracetamol (2g/kg, p.o)</td>
<td>4.96 ± 0.73</td>
</tr>
<tr>
<td>Silymarin (100mg/kg/day, p.o.) + PCM</td>
<td>2.64 ± 0.18**</td>
</tr>
<tr>
<td>AC (200mg/kg/day, p.o.) + PCM</td>
<td>3.2 ± 0.24**</td>
</tr>
<tr>
<td>AC (400mg/kg/day, p.o.) + PCM</td>
<td>2.70 ± 0.16**</td>
</tr>
<tr>
<td>LC (200mg/kg/day, p.o.) + PCM</td>
<td>2.93 ± 0.14**</td>
</tr>
<tr>
<td>LC (400mg/kg/day, p.o.) + PCM</td>
<td>2.68 ± 0.30**</td>
</tr>
</tbody>
</table>

n=6, Values were expressed as mean ± SEM *P<0.01 Vs Paracetamol, significant; **P<0.001 highly significant. Data were analyzed by One way ANOVA followed by Dunnett’s ‘t’ test.
RESULTS

Paracetamol increased the liver weight to 4.96±0.73 g from 2.51±0.42 g as comparable to the control. AC 200 mg, 400 mg and LC 200 mg, 400 mg reduced the liver weights to 3.2±0.24 g (p<0.001), 2.70±0.16 g (p<0.001), 2.93±0.14 g (p<0.001) and 2.68±0.30 g (p<0.001) respectively as comparable to the toxic. AC and LC produced statistically significant dose dependent hepatoprotective activity but less than the standard, Silymarin (100 mg), 4.2±0.062 g (p<0.001).
Table No 37: Results of AC and LC on average liver weight of treated animals against D-Galactosamine

<table>
<thead>
<tr>
<th>Design of treatment</th>
<th>Liver weight g/100g of body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Vehicle 1ml/kg/day,p.o)</td>
<td>2.51 ± 0.42</td>
</tr>
<tr>
<td>D-Galactosamine (400mg/kg)</td>
<td>6.03 ± 0.07</td>
</tr>
<tr>
<td>Silymarin (100mg/kg/day,p.o.) + D-Galactosamine</td>
<td>3.22 ± 0.062**</td>
</tr>
<tr>
<td>AC (200mg/kg/day,p.o.) + D-Galactosamine</td>
<td>3.64 ± 0.05**</td>
</tr>
<tr>
<td>AC(400mg/kg/day,p.o.) + D-Galactosamine</td>
<td>3.21 ± 0.07**</td>
</tr>
<tr>
<td>LC (200mg/kg/day,p.o.) + D-Galactosamine</td>
<td>3.75 ± 0.36**</td>
</tr>
<tr>
<td>LC (400mg/kg/day,p.o.) + D-Galactosamine</td>
<td>3.42 ± 0.23**</td>
</tr>
</tbody>
</table>

n=6, Values were expressed as mean ± SEM *P<0.01 Vs D-Galactosamine, significant; **P<.0.001 highly significant. Data were analyzed by One way ANOVA followed by Dunnett’s ‘t’ test.
RESULTS

D-Galactosamine increased the liver weight to 6.03±0.07 g from 2.51 ± 0.42g as comparable to the control. AC 200 mg, 400 mg, and LC 200 mg, 400 mg reduced the liver weights to 4.6±0.05 g (p<0.001), 3.4 ± 0.07g (p<0.001), 5.4 ± 0.36g (p<0.001) and 3.2 ± 0.23g (p<0.001) respectively as comparable to the toxic. AC and LC produced statistically significant dose dependent hepatoprotective activity but less than the standard, Silymarin (100 mg), 4.2±0.062 g (p<0.001).
Table No 38: Results of AC and LC on average liver weight of treated animals against Thioacetamide

<table>
<thead>
<tr>
<th>Design of treatment</th>
<th>Liver weight (g) /100g of body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Vehicle 1ml/kg/day, p.o)</td>
<td>2.51 ± 0.42</td>
</tr>
<tr>
<td>Thioacetamide (100mg/kg, i.p)</td>
<td>3.99 ± 0.28</td>
</tr>
<tr>
<td>Silymarin (100mg/kg/day, p.o.) + TAA</td>
<td>2.54 ± 0.61**</td>
</tr>
<tr>
<td>AC (200mg/kg/day, p.o.) + TAA</td>
<td>3.31 ± 0.36**</td>
</tr>
<tr>
<td>AC (400mg/kg/day, p.o.) + TAA</td>
<td>2.07 ± 0.02**</td>
</tr>
<tr>
<td>LC (200mg/kg/day, p.o.) + TAA</td>
<td>3.81 ± 0.26**</td>
</tr>
<tr>
<td>LC (400mg/kg/day, p.o.) + TAA</td>
<td>2.66 ± 0.41**</td>
</tr>
</tbody>
</table>

n=6, Values were expressed as mean ± SEM *P<0.01 Vs Thioacetamide, significant; **P<0.001 highly significant. Data were analyzed by One way ANOVA followed by Dunnett’s ‘t’ test.

Fig: 38 Results of AC and LC on average liver weight of treated animals against Thioacetamide
RESULTS

Thioacetamide increased the liver weight to 4.96±0.73 g from 2.51±0.42 g as comparable to the control. AC 200 mg, 400 mg and LC 200 mg, 400 mg reduced the liver weights to 3.2±0.24 g (p<0.001), 2.70±0.16 g (p<0.001), 2.93±0.14 g (p<0.001) and 2.68±0.30 g (p<0.001) respectively as comparable to the toxic. AC and LC produced statistically significant dose dependent hepatoprotective activity but less than the standard, Silymarin (100 mg), 4.2±0.062 g (p<0.001).

Table No 39: Results of AC and LC on average liver weight of treated animals against Rifampicin

<table>
<thead>
<tr>
<th>Design of treatment</th>
<th>Liver weight (g)/100g of body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Vehicle 1ml/kg/day,p.o)</td>
<td>2.51 ± 0.42</td>
</tr>
<tr>
<td>Rifampicin (100mg/kg, p.o)</td>
<td>4.81 ± 0.26</td>
</tr>
<tr>
<td>Silymarin (100mg/kg/day,p.o.) + RMP</td>
<td>2.06 ± 0.71**</td>
</tr>
<tr>
<td>AC (200mg/kg/day,p.o.) + RMP</td>
<td>3.12 ± 0.13**</td>
</tr>
<tr>
<td>AC (400mg/kg/day,p.o.) + RMP</td>
<td>2.50 ±0.25**</td>
</tr>
<tr>
<td>LC (200mg/kg/day,p.o.) + RMP</td>
<td>2.85 ± 0.53**</td>
</tr>
<tr>
<td>LC (400mg/kg/day,p.o.) + RMP</td>
<td>2.59 ± 0.71**</td>
</tr>
</tbody>
</table>

n=6, Values were expressed as mean ± SEM *P<0.01 Vs Rifampicin, significant; **P<0.001 highly significant. Data were analyzed by One way ANOVA followed by Dunnett’s’t’ test.
Fig: 39 Results of AC and LC on average liver weight of treated animals against Rifampicin

RESULTS

Rifampicin increased the liver weight to 4.96±0.73 g from 2.51±0.42 g as comparable to the control. AC 200 mg, 400 mg and LC 200 mg, 400 mg reduced the liver weights to 3.2±0.24 g (p<0.001), 2.70±0.16 g (p<0.001), 2.93±0.14 g (p<0.001) and 2.68±0.30 g (p<0.001) respectively as comparable to the toxic. AC and LC produced statistically significant dose dependent hepatoprotective activity but less than the standard, Silymarin (100 mg), 4.2±0.062 g (p<0.001).
6.6.2 HISTOPATHOLOGICAL STUDIES OF LIVER

A part of liver was subjected for histopathology and remaining was subjected for anti-oxidants studies.

The liver was examined grossly and a part of it of each animal was fixed in 10% buffered neutral formalin for 48 hour and then with bovine solution for 6 hour. Paraffin sections were taken at 5 µm thickness processed in alcohol-xylene series and was stained with alum hematoxylin and eosin. The section was examined microscopically for histopathological changes. The same procedure was carried out for all animals.

The isolated liver was sliced into 5 mm pieces and fixed in neutral formalin (10 %) solution for 3 days and washed under running water for about 12 hrs. This was followed by dehydration with alcohol of increasing strength (70%, 80%, and 90%) for 12 hrs each. Final dehydration was carried out using absolute alcohol with about 3 changes at 12 min interval. Cleaning was done by using xylin with changes at 15 – 20 min interval. After cleaning the pieces were subjected to paraffin infiltration in automatic tissue processing unit. The pieces were washed under running water to remove formalin completely. The following steps were performed,

**a. Embedding in paraffin**

Hard paraffin was melted and poured into L-shaped block. The tissue pieces were then dropped into the liquid paraffin quickly and allowed to cool.

**b. Sectioning**

The blocks were cut using microtone to get sections of thickness of 5 microns. The section were fixed on a glass using albumin and allowed to dry.

**c. Staining**

Eosin an acid stain and hematoxylin, a basic stain were used for staining the liver sections.
d. Mounting

The section was then mounted in diphenyl xylin. Staining result showed blue colour nucleus and cytoplasm with various shade of pink with change in different tissue component. The prepared slides were observed under light microscope for their histological details and photographs were taken. (Pantin, et al., 1962).

The results were represented in figures 48-55
A. STUDY ON HEPATOPROTECTIVE ACTIVITY OF *ACALYPHA COMMUNIS* AND *LINDERA COMMUNIS* AGAINST PARACETAMOL INDUCED

Fig: 40 Histopathological Studies of liver (Paracetamol induced)
B. STUDY ON HEPATOPROTECTIVE ACTIVITY OF *ACALYPHA COMMUNIS* AND *LINDERA COMMUNIS* AGAINST D-GALACTOSAMINE INDUCED

Fig: 41 Histopathological Studies of liver (D-GalN induced)
C. STUDY ON HEPATOPROTETIVE ACTIVITY OF *ACALYPHA COMMUNIS* AND *LINDERA COMMUNIS* AGAINST THIOACETAMIDE INDUCED

Fig. 42 Histopathological Studies of liver (Thioacetamide induced)
D. STUDY ON HEPATOPROTECTIVE ACTIVITY OF ACALYPHA COMMUNIS AND LINDERA COMMUNIS AGAINST RIFAMPICIN INDUCED

Fig: 43 Histopathological Studies of liver (Rifampicin induced)
7.2.1 Histopathological Studies of liver (Paracetamol induced)

**Group I-** Normal control (NaCl 0.9% w/v) (5ml/kg)

Section showed liver. The architecture is maintained. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval uniform nuclei.

**Group II-** Paracetamol (2g/kg)

Section showed liver with feathery degeneration and focal necrosis. The architecture was mildly distorted. The central veins were congested. There was patchy necrosis of the hepatocytes at focal areas. The portal tracts showed mild chronic inflammation composed of lymphocytes.

**Group III-** Silymarin + Paracetamol (100mg/kg) + (2g/kg)

Section showed liver. The architecture was normal. The central veins appeared normal. The hepatocytes showed round uniform nuclei and moderate cytoplasm. The portal triads showed mild periportal inflammation composed of lymphocytes.

**Group IV -** AC+ Paracetamol (200mg/kg) + (2g/kg)

Section showed liver with patchy hepatocyte necrosis. The central veins were normal. The portal triads showed mild periportal inflammation composed of lymphocytes.

**Group V -** AC+ Paracetamol (400mg/kg) + (2g/kg)

Section showed liver. The portal triads were normal. The hepatocytes appeared normal and showed moderate cytoplasm and round to oval nuclei. There was some degeneration of the hepatocytes.

**Group VI -** LC+ Paracetamol (200mg/kg) + (2g/kg)

Section showed liver with partially effaced architecture. The central veins are dilated and congested. The hepatocytes showed fatty steatosis. The portal triads showed periportal inflammation composed of lymphocytes.

**Group VII -** LC+ Paracetamol (400mg/kg) + (2g/kg)
Section showed liver. The architecture was mildly distorted. The portal triads were normal. The hepatocytes showed mild feathery degeneration. The portal triads appeared normal.

7.2.2 Histopathological Studies of liver (D-Galactosamine induced)

**Group I**- Normal control (NaCl 0.9% w/v) (5ml/kg)

Section showed liver. The architecture is maintained. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval nuclei.

**Group II**- D-Galactosamine (400mg/kg p.o)

Section showed liver with distorted architecture. The central veins were normal. There is patchy necrosis of the hepatocytes at focal areas. The portal tracts showed mild chronic inflammation composed of lymphocytes.

**Group III**- Silymarin + D-Galactosamine (100mg/kg) +(400mg/kg)

Section showed liver. The architecture was normal. The central veins showed mild congestion. The hepatocytes were normal and showed moderate cytoplasm and round uniform nuclei. The portal triads are normal.

**Group IV** - AC+ D-Galactosamine  (200mg/kg) +(400mg/kg)

Section showed liver with normal architecture. The central veins are dilated and congested. The hepatocytes show fatty steatosis. The portal triads appear normal.

**Group V** - AC+ D-Galactosamine  (400mg/kg) +(400mg/kg)

Section showed liver. The architecture was normal. The portal triads were normal. The hepatocytes appeared normal and showed moderate cytoplasm and round to oval nuclei. There was no feathery degeneration of the hepatocytes.

**Group VI** - LC+ D-Galactosamine  (200mg/kg) +(400mg/kg)
Section showed liver with normal architecture. The central veins were congested. The hepatocytes showed feathery degeneration. The portal triads showed periportal inflammation composed of lymphocytes.

**Group VII** - LC+ D-Galactosamine (400mg/kg) +(400mg/kg)

Section showed liver. The architecture was mildly distorted. The portal triads show inflammation. The hepatocytes appeared normal and showed moderate cytoplasm and round to oval nuclei. There is no feathery degeneration of the hepatocytes.

### 7.2.3 Histopathological Studies of liver (Thioacetamide induced)

**Group I**- Normal control (NaCl 0.9% w/v) (5ml/kg)

Section showed liver. The architecture was maintained. The central veins, sinusoids and portal triads appeared normal. The hepatocytes showed moderate cytoplasm and round to oval nuclei.

**Group II**- Thioacetamide (100mg/kg i.p)

Showed moderate to severe liver damage characterized by clear cytoplasm, vascular congestion, fatty changes, apoptosis and focal areas of necrosis and vacuolation of cytoplasm as a feature of ballooning degeneration.

**Group III**- Silymarin (100mg/kg) + Thioacetamide (100mg/kg i.p)

Showed normal liver architecture and occasional inflammatory cells with no necrosis. Microscopically normal lobular appearance having normal central vein

**Group IV** – AC (200mg/kg) + Thioacetamide (100mg/kg i.p)

Liver specimen of cirrhotic liver showing regenerating hepatocytes starting the periphery

**Group V** – AC (400mg/kg) + Thioacetamide (100mg/kg)

Normal hepatic lobule architecture was seen. Hepatocytes and their nuclei were well visible. Few inflammatory cells were seen around central veins. Necrosis was absent.

**Group VI** – LC (200mg/kg) + Thioacetamide (100mg/kg)
Liver specimen of cirrhotic liver showing marked regeneration of hepatic lobules with no evidence fibrosis of portal tracts

**Group VII** – LC (400mg/kg) + Thioacetamide (100mg/kg)

Liver specimen of liver showing marked regeneration of hepatic lobules with no normal portal tracts

### 7.2.4 Histopathological Studies of liver (RMP induced)

**Group I**- Normal control (NaCl 0.9% w/v) (5ml/kg)

Section showed liver. The architecture was maintained. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval nuclei.

**Group II**- RMP (100mg/kg p.o)

Liver specimen showed liver cirrhosis and marked portal tracts fibrosis and inflammatory infiltrate

**Group III**- Silymarin (100mg/kg) + RMP (100mg/kg p.o)

Section of liver tissue of rat treated with silymarin showing almost normal liver architecture with no necrosis, no steatosis and mild sinusoidal congestion

**Group IV** – AC (200mg/kg) + RMP (100mg/kg p.o)

Section of liver tissue of rat treated showing almost abnormal liver architecture with no necrosis, but less steatosis and mild sinusoidal congestion

**Group V** – AC (400mg/kg) + RMP (100mg/kg p.o)

Showed normal gross appearance having smooth surfaces, microscopically normal lobular appearance having normal central vein, radiating cords of hepatocyte and normal portal tract.

**Group VI** – LC (200mg/kg) + RMP (100mg/kg p.o)

Showed normal liver architecture and occasional inflammatory cells with no necrosis
Group VII – LC (400mg/kg) + RMP (100mg/kg p.o)

Showed normal architecture, granulated cytoplasm and uniform nuclei and less disruption and deterioration of hepatocytes