Protection Role of *Luffa cylindrica* Linn against Erythromycin Estolate Induced Hepatotoxicity.

Shete R.V., Pawashe P. M., Kore K. J., Otari K.V.
Rajgad Dyanpeeth’s College of Pharmacy, Bhor, Dist. Pune- 412206, Maharashtra, India.

**Abstract**
*Luffa cylindrica* Linn (LC) was examined to justify its role in the hepatoprotection against erythromycin toxicity in male rats. Oral daily administration of toxic dose of erythromycin estolate (EE, 100 mg/kg) was given to rats for fourteen days to induce hepatotoxicity. It was found that physical parameters like liver weight, liver volume markedly increased in rat treated with erythromycin estolate (EE). The hepatotoxicities were monitored by increased level of Serum enzymes like Serum glutamate pyruvate transaminase (SGPT) and Serum Glutamic Oxaloacetic transaminase (SGOT), Alkaline Phosphatase (ALP) & total bilirubin (TBL). The data obtained showed that oral administration of Hydroalcoholic extract of leaves of *Luffa cylindrica* Linn (HELC 250 mg/kg, 500 mg/kg & 1000 mg/kg body weight) was significantly prevented the occurrence of EE-induced liver damage. Silymarin (50 mg/kg) was given as a reference standard. The hepatic antioxidant status such as lipid peroxidation measured as MDA level showed increase and glutathione activities were reduced in the EE treated animals. Administration of HELC restored the hepatic antioxidant status. The biochemical data were supplemented by histopathological examination of the liver of control and treated rats. Studies showed that LC will be a prophylactic drug in treating the conditions of hepatic disorders.

**Key Words**
*Luffa cylindrica*, erythromycin estolate, hepatotoxicity, antioxidant.

**Introduction**
Liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, metabolism, fight against disease, nutrient supply, energy provision and reproduction. In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. In experimental hepatotoxicity models in laboratory or higher animals, several herbals exerted hepatoprotective/curative effects that warrants their clinical testing. Due to lack of scientific based pharmacological data, most of the herbal formulations cannot be recommended for the treatment of liver diseases. Leaves and stems of an herb *Luffa cylindrica* Linn. (LC) are reported to be traditionally used in liver diseases. The phytoconstituents, flavonoids and triterpenoids present in the plant are well known for their antioxidant and hepatoprotective activities. It also has been proved especially for its antioxidant property. Its anti-radical activity is responsible for its anti-oxidant action.

Considering above properties and chemical constituents present in the plant, the present study was undertaken to explore the utility of hydroalcoholic extract of *Luffa cylindrica* leaves as liver protective and antioxidant.

**Collection and extraction**
The leaves of LC were collected from Bhor region, Dist. Pune, India in the month of September, 2009 and authenticated from Yashwantrao Chavan Institute of Sciences, Karad, Dist. Satara, Maharashtra and herbarium was deposited (Number Y.C.S.K./bot./11). Fresh leaves were collected, shade dried and coarsly powdered. The powdered material was macerated for 24 h in ethanol 50 % v/v then it was subjected to percolation for 24 h. The menstrum was collected and evaporated for drying by heating on water bath. After drying dark green coloured semisolid mass was obtained which was used for further experimental procedures.

**Experimental animals**
Swiss albino mice weighing between 18-25 gm and wistar rats weighing between 215-260 gm of either sex were procured from National Institute of Bioscience, Pune, Maharashtra, India. The animals...
Protective Role of Luffa cylindrica Linn against Erythromycin Estolate

Pawashe P M et al

were housed under standard husbandry conditions (25±2°C, relative humidity of 45-55 %) for 12-h: 12-h light: dark cycle respectively and were given standard food and water Ad. libitum. All the experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Rajgad Dnyanpeeth’s College of Pharmacy, Bhor, Pune, Maharashtra, India (Approval No. RDCOP/IAEC/10/07).

Acute Oral toxicity Studies
The acute oral toxicity study was performed as per OECD guideline 423. The administration of HELC at limit dose of 5000 mg/kg did not showed any signs and symptoms of any toxicity in the animals. At this dose no mortality and morbidity was observed during the 14 days of observational period. Hence doses 250 mg/kg, 500 mg/kg and 1000 mg/kg of HELC were selected for present study.

Experimental Protocol
Male wistar rats of (215–260 g) were divided into six groups (n=6) and treated as follows. At the end of 14 days, the animals were sacrificed by cervical dislocation. Blood was collected from the heart. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated and used for the estimation of various biochemical parameters such as SGOT, SGPT, ALP and TBL. After collection of blood samples, the rats were sacrificed and their livers were excised and the net liver weight and volume, antioxidant & histopathological examination was carried out.

Physical parameters
Liver weight of each animals of treatment groups are taken on electronic balance. Liver volumes were estimated by water displacement method.

Biochemical parameters
Biochemical parameter i.e., Serum glutamate pyruvate transaminase (SGPT), Serum glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP), Bilirubin, were analyzed according standard kits.

Antioxidant parameters in tissue
Lipid peroxidation measured as MDA level was carried out by the method of Ilavarasan et al., 2005 & Ohakawa et al., 1979. Estimation of glutathione was carried out by Orhan et al., 2003.

Histopathological examination
A portion of the liver was cut into two to three pieces of approximately 6 mm³ size and fixed in phosphate buffered 10% formaldehyde solution. After embedding in paraffin wax, thin sections of liver tissue were cut and stained with haematoxylineosin. The thin sections of liver were made into permanent slides and examined under high resolution microscope with photographic facility and photomicrographs are taken.

Statistical analysis
The arithmetic mean±SEM values were calculated for each experimental group. The results were analyzed by using student t-test and One-Way ANOVA followed by Dunnet’s test. P<0.05 and p<0.01 were considered to be statistically significant.

Results and Discussion
Erythromycin estolate was reported to be metabolised to reactive nitrosoalkane derivatives, which may be further metabolised to reactive nitroso radicals. Nitrosoalkane derivative which binds covalently to thiol groups of proteins and other cellular macromolecules. During the course of aerobic metabolic reactions, considerable amounts of reactive oxygen species such as superoxide anion (O₂⁻) and hydrogen peroxide (H₂O₂) are generated which undergo a variety of chain reactions and produce free radicals such as OH’. These hydrogen species attack polyunsaturated fatty acids and thereby initiate the process of lipid peroxidation resulting in oxidative degradation and inactivation of various important biomolecules. When the liver cell plasma membrane is damaged, varieties of enzyme normally located on the cytosol are released into the bloodstream. Their estimation in serum is a useful quantitative marker of the extent and type of hepatocellular damage. The increased activities of these serum markers observed extensive liver damage induced in rats treated with erythromycin estolate. Treatment with HELC and Silymarin 50 mg/kg/day significantly reduced the SGOT, SGPT, ALP and bilirubin levels, suggesting that they offered protection by preserving the structural integrity of the hepatocellular membrane against...
Protective Role of *Luffa cylindrica* Linn against Erythromycin Estolate

Pawashe P M et al

In the present study, the increased levels of lipid peroxidation measured as MDA level reflected as the consequence of oxidative stress caused by erythromycin estolate. Treatment with HELC offered protection through attenuation of lipid peroxidation and decreased production of free-radical derivatives, as evident from the decreased levels of MDA. Treatment with HELC offered protection to cells against oxidative stress by scavenging free radicals. Silymarin also significantly reduced lipid peroxidation by its ability to inhibit oxidative damage. GSH being the most important biomolecule against chemically induced toxicity can participate in the elimination of reactive intermediates by reduction of hydroperoxides in the presence of GSH-dependent enzymes. GSH also functions as a free radical scavenger and in the repair of radical caused biological damage17. In the present study, the level of GSH was markedly increased by treatment with HELC and Silymarin. Increased level of the GSH would therefore be important in protecting against erythromycin estolate toxicity. Administration of erythromycin estolate (100mg/kg/day; p.o.), showed histopathological injury to liver tissue as evident from necrosis, hepatocellular damage and degeneration in liver sections of the EE control as compared with liver sections of normal control. Treatment with HELC (250, 500 and 1000 mg/kg/day; p.o.) showed reduction in the central necrosis, hepatocyte degeneration and inflammation. Treatment with silymarin 50 mg/kg showed normal liver lobule with no signs of necrosis.

**Conclusion**

Hence LC will be a prophylactic drug in treating the conditions of hepatic disorders. The results of present study were suggested that LC may possibly acts as antioxidant in preventing the conditions of hepatic disorders. Further studies are required to evaluate the mechanism of action of *Luffa cylindrica* (L.) Roem.

**References**


---

**Fig No. 1:** Photomicrograph of liver from animals treated with a) Normal control b) EE control c) Silymarin-50 d) HELC-250 e) HELC-500 f) HELC-1000. (H. and E. 400x) (EE-Erythromycin estolate).
Table No. 1: Treatment schedule.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Group</th>
<th>Treatments</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>Vehicle 10 ml/kg, (p.o.)</td>
<td>14 days</td>
</tr>
<tr>
<td>II</td>
<td>EE control</td>
<td>Erythromycin estolate 100 mg/kg, (p.o.)</td>
<td>14 days</td>
</tr>
<tr>
<td>III</td>
<td>Silymarin 50</td>
<td>50 mg/kg/day, (p.o.)</td>
<td>14 days</td>
</tr>
<tr>
<td>IV</td>
<td>HELC 250</td>
<td>250 mg/kg/day, (p.o.)</td>
<td>14 days</td>
</tr>
<tr>
<td>V</td>
<td>HELC 500</td>
<td>500 mg/kg/day, (p.o.)</td>
<td>14 days</td>
</tr>
<tr>
<td>VI</td>
<td>HELC 1000</td>
<td>1000 mg/kg/day, (p.o.)</td>
<td>14 days</td>
</tr>
</tbody>
</table>

Table No. 2: Hepatoprotective activity of hydroalcoholic extract of leaves of *Luffa cylindrica* Linn on physical, serum, tissue antioxidant parameters.

<table>
<thead>
<tr>
<th>Groups (mg/kg)</th>
<th>Liver weight (g/100 g)</th>
<th>Liver volume (ml/100 g)</th>
<th>SGOT (IU/l)</th>
<th>SGPT (IU/l)</th>
<th>ALP (U/l)</th>
<th>TBL mg%</th>
<th>MDA (nmol/g)</th>
<th>GSH (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>3.82± 0.17</td>
<td>63.95± 2.12</td>
<td>22.99± 1.79</td>
<td>72.68± 2.11</td>
<td>0.63± 0.041</td>
<td>206.7± 4.56±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EE control</td>
<td>6.18± 0.27#</td>
<td>95.1± 2.48#</td>
<td>44.59± 1.85#</td>
<td>116± 2.17#</td>
<td>1.45± 0.10#</td>
<td>381.3± 2.49±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silymarin 50</td>
<td>5.10± 0.11**</td>
<td>80.93± 3.54**</td>
<td>24.88± 1.36**</td>
<td>94.8± 1.94**</td>
<td>1.45± 0.02**</td>
<td>202.8± 4.94±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HELC 250</td>
<td>5.32± 0.26*</td>
<td>82.63± 3.09*</td>
<td>32.23± 5.18</td>
<td>108.8± 1.28±</td>
<td>0.04 18.36</td>
<td>311.9± 3.227±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HELC 500</td>
<td>5.59± 0.21</td>
<td>81.88± 2.83*</td>
<td>33.67± 2.38*</td>
<td>105.5± 2.93</td>
<td>1.09± 0.06*</td>
<td>239.9± 3.342±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HELC 1000</td>
<td>5.13± 0.23**</td>
<td>82.1± 2.28*</td>
<td>31.97± 2.84*</td>
<td>97.83± 3.40**</td>
<td>1.09± 0.09*</td>
<td>245.4± 4.338±</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM (n=6). # p<0.01 as compared with normal control (Student t-test). *p<0.05 & **p<0.01 as compared with EE control group (one-way ANOVA followed by Dunnett’s test).

*******