ABSTRACT
The hydroalcoholic extract of whole plant of Acalypha indica (AI) was evaluated for its hepatoprotective activity against CCl₄ and Rifampicin - Isoniazid combination induced hepatotoxicity at two dose levels 150 and 300 mg kg⁻¹. Acalypha indica (AI) exhibited a significant protective action on the liver evident by a reduction in the elevated levels of serum lysosomal enzymes namely Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP) in both CCl₄ and Rifampicin-Isoniazid induced hepatotoxicity. The hepatoprotective activity of Acalypha indica was evaluated for possible potentiating in the presence of piperine based on recent research which has reported the latter enhancing bioavailability of certain drugs and nutritional compounds. Piperine was checked for potentiation, if any, at two dose levels 10 and 20 mg, respectively. Piperine and AI treatment on biochemical parameters in Rifampicin-Isoniazid induced hepatotoxicity, All values are expressed as Mean ±SEM, N = 6. Analysis by one way ANOVA followed by Dunnett’s test. *Significant at p<0.05, **Significant at p<0.01 in which all values are compared against toxicant control group. The toxicant control group is compared against the control group dose dependent potentiation of the hepatoprotective activity of Acalypha indica

Keywords: Hepatoprotective activity, Rifampicin-Isoniazid, SGOT, SGPT, ALP Acalypha indica.

INTRODUCTION
Liver has a pivotal role in the regulation of physiological processes. It is involved in several vital functions such as storage, secretion, metabolism and detoxification of a variety of drugs and xenobiotics. Liver diseases are mainly caused by either toxic chemicals (certain antibiotics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons etc.) excess consumption of alcohol, infections or autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver (Atal, C.K et al). Though liver diseases are amongst the important diseases affecting mankind, no remedy is available to majority of them at present Acalypha indica (Euphorbiaceae) in the Indian system of medicine, is a perennial creeping herb found throughout India. The whole plant are reputed to be it is useful in bronchitis, asthma, pneumonia, laxative and jaundice. Leaves and twigs contain alkaloids like acalyphine, acalyphamide, amides, quinine, sterols and cyanogenic glycosides. Plant contains kaemperol, sitosterol, triacetamino (Bano, G., et al) and new amides like auranthiamide and its acetate succinimide, 2-methylanthraquinone, tri-o-methylellagic acid were isolated from leaves of Acalypha indica Linn. (Bhalla T.N et al). The extract of whole plant was reported for post coital antifertility activit (Bhupinder, S.K, et al) the aqueous extract of Acalypha indica Linn. Shown neuroprotection and neurotherapy effect (Chakraborti, K.K et al) extract of leaves has shown antibacterial activity (Ellard, G.A et al) and anti-inflammatory activity (Jain, G.K et al), different
extracts of *Acalypha indica* Linn. shown Larvicidal and ovicidal activity against *Anopheles stephensi* (Juvekar, A.R et al., and Antibacterial activity of biosynthesized silver nanoparticles from *Acalypha indica* Linn. has been reported (Lal, S. et al.). Only few plants are really very promising hepatoprotective agents. Realizing the importance and common use of the whole plant of *Acalypha indica* in the treatment of liver disorders by several tribes in India, it was decided to investigate the hepatoprotective activity of *Acalypha indica* whole plant. Piperine is reported to increase the bioavailability of valuable phytochemicals present in other species and can boost the activity of biochemically active compounds such as phenytoin (Misra, A.N. et al) co-enzyme Q (Nandave, M et al.), beta-carotene (Rafatullah, S et al) curcumin (Recknagel, R.O et al) and a variety of other spices by up to several hundred percent, depending on the molecule concerned. Since no effective treatment has been established for liver disorders, it is very crucial to find out the manner in which liver can be best treated. The possibility of enhancing the existing activity of *Acalypha indica* with piperine thereby increasing its efficacy might open new vistas in the treatment of liver disorders. In general, such an approach would ensure increase in efficacy of herbal formulations used to treat oxidative stress in the liver and also help combat tuberculosis more effectively which has remained a life threatening disease. An attempt was hence made to evaluate the hepatoprotective activity of roots of *Acalypha indica* and subsequently investigate the effect of piperine, if any, on its bioactivity.

**MATERIALS AND METHODS**

**Animals:** Albino Wistar rats of either sex, in the weight range of 180-200 g, maintained on natural light/dark cycle, at a temperature of 25 ±2 °C, commercial pellet diet and water *ad libitum* were used in the study. Experimental protocols for the pharmacological and toxicity studies were reviewed and approved by the Institutional Animal Ethics Committee (Animal House Registration No. 23/2001/CPCSEA) which conforms to the Indian National Science Academy Guidelines for the use and care of experimental animals in research.

**Plant materials and drugs:** whole plant of *Acalypha indica* were collected from Local area of Warangal, authenticated by Dr.Rju kakatiya university, Warangal AP. November 2011, coarsely powdered in a micro-pulverized and subjected to Sox let extraction, using a hydro alcoholic (70% ethanol) solvent at a temperature of 70 °C. The extract was concentrated by drying it over a water bath at a temperature of 70 °C. Silymarin obtained from Sigma Aldrich, Mumbai, was used as a reference standard for this study. Rifampicin and Isoniazid were obtained as gift samples from Mc leods Pharmaceuticals and Ajanta Pharma Ltd., Mumbai, respectively. All serum marker enzyme estimations were carried out using the Cobas Integra- 400 auto-analyzer at Acute laboratories, Navi Mumbai with the help of standard diagnostic kits from Roche diagnostics. All reagents and chemicals used were of analytical grade.

**EXPERIMENTAL**

**Effect of Piperine on CCl4 induced hepatotoxicity** (Rafatullah, S., A et al) (Shenoy, K.A et al): Forty two rats of either sex were divided into 7 groups containing 6 rats each:

**Group 1:** Control group which was administered distilled water 1 mL kg<sup>-1</sup> i.p. for 10 days.

**Group 2:** Toxicant control group which was administered the toxicant CCl4 in olive oil (1:1)-1 mL kg<sup>-1</sup> i.p. from the 4th to the 10th day.

**Group 3:** Standard group which was administered the standard Silymarin-100 mg kg<sup>-1</sup> p.o. for 10 days, an hour before administration of the toxicant-CCl4 in olive oil (1:1)-1 mL kg<sup>-1</sup> i.p. from the 4th to the 10th day.

**Group 4:** Treatment group receiving Al at 150 mg kg<sup>-1</sup> p.o. for 10 days, an hour before administration of the toxicant-CCl4 in olive oil (1:1)-1 mL kg<sup>-1</sup> i.p. from the 4th to the 10th day.

**Group 5:** Treatment group receiving Al at 300 mg kg<sup>-1</sup> p.o. for 10 days, an hour before administration of the toxicant-CCl4 in olive oil (1:1)-1 mL kg<sup>-1</sup> i.p. from the 4th to the 10th day.

**Group 6:** Potentiation group which was administered 10 mg piperine concomitantly with 150 mg kg<sup>-1</sup> of Al p.o. for 10 days, an hour before administration of the toxicant- CCl4 in olive oil (1:1)-1 mL kg<sup>-1</sup> i.p. from the 4th to the 10th day.

**Group 7:** Potentiation group which was administered 20 mg piperine concomitantly with 150 mg kg<sup>-1</sup> of Al p.o. for 10 days, an hour before administration of the toxicant-CCl4 in olive oil (1:1)-1 mL kg<sup>-1</sup> i.p. from the 4th to the 10th day.

**Duration of study:** Ten days.
**Procedure:** The rats were sacrificed on the 11th day under light ether anaesthesia. Blood withdrawal (4 mL) was carried out by cardiac puncture; serum was separated by centrifugation at 3000 rpm using the pathological centrifuge. Estimation of biochemical parameters namely serum marker enzymes - SGOT, SGPT, ALP were done by using the auto-analyzer.

**Statistical analysis:** All the values are expressed as Mean ±SEM and data analyzed by one-way ANOVA, using Graphpad INSTAT. The level of significance was found out by Dennett's test wherein all the groups are compared against control. *p<0.01 was considered to be significant.

**RESULTS**
In CCl₄ induced hepatotoxicity the administration of the toxicant CCl₄ showed a distinct rise in the levels of serum marker enzymes namely SGOT, SGPT and ALP as shown in Table 2. The drug treatment (AI) was carried out at 2 dose levels 150 and 300 mg kg⁻¹ both of which along with the standard (silymarin) treated group showed a significant reduction in the elevated enzyme levels (p<0.01). However the treatment group at 300 mg kg⁻¹ showed values of enzymes comparable to the control group as shown by the statistical analysis (p>0.01). Taken together these data suggest a dose dependent hepatoprotective activity of AI.

In Rifampicin-Isoniazid induced toxicity (Table 2) a considerable elevation in the levels of serum enzymes was observed in group 2 receiving only toxicant i.e., Rifampicin and Isoniazid. The enzyme levels of the standard silymarin treated group were found to be considerably reduced (p<0.01) and comparable to control. However the drug treatment at 150 mg kg⁻¹ showed that only the reduction in the ALP levels were found to be significant. In comparison the drug treatment at 300 mg kg⁻¹ also showed statistically significant reduction in levels of enzymes comparable to control with (p<0.01). Like CCl₄ these data also suggest dose dependent hepatoprotective activity of AIE. It shows the potentiation of AI activity by piperine in CCl₄ induced hepatotoxicity which was found to be dose dependent with both groups showing significant reduction in the enzyme levels (p<0.01). In Rifampicin-Isoniazid induced hepatotoxicity, piperine -10 mg was found to be quite significant (p<0.01) for reduction in the ALP levels and the group but less significant (p<0.05) for SGOT and SGPT. However piperine -20 mg was found to be extremely significant with a marked reduction in levels of all enzymes (p<0.01). Thus the above data suggests that piperine was found to potentiate the hepatoprotective activity of AI in a dose dependent manner.

**DISCUSSION**
Carbon tetrachloride induced hepatotoxicity is well documented regarding its toxic effects on the liver.
Following CCl₄ administration the toxin CCl₄ is biotransformed by cytochrome P-450 to produce the trichloromethyl free radical (CCl₃⁺). This is turn elicits lipid peroxidation of membrane lipids in the presence of oxygen radical generated by metabolic leakage from mitochondria. All these events culminate in functional and morphological changes leading to loss of integrity of cell membranes which is evidenced by the rise in levels of serum marker enzymes- SGOT, SGPT, ALP, damage of hepatic tissue due to the reduced activity of the anti-oxidant enzymes and disturbance of Ca²⁺ homeostasis (Velpandian T.,R et al.). SGPT is an enzyme present in hepatocytes (liver cells). When a cell is damaged, it leaks this enzyme into the blood, where it is measured. SGPT rises dramatically in acute liver damage. SGOT is similar to SGPT in that it is another enzyme associated with liver parenchyma cells. It is raised in acute liver damage but also present in red cells, cardiac and skeletal muscle and is therefore not specific to the liver (Vladimir, B et al). ALP is present in cells lining the biliary ducts. ALP levels rise with infiltrative diseases of the liver. As evidenced in Table 1 and 2 the treatment groups lower the levels of SGPT, SGOT and ALP which are observed to be higher in the toxicant groups thus indicating damage to the hepatocellular membrane.

The use of isoniazid (INH) and rifampicin in the treatment of tuberculosis is limited by their potential for hepatotoxicity. The incidence of hepatotoxicity is higher with isoniazid and rifampicin combination than with isoniazid or rifampicin alone (Vladimir, B. and M. Majeed, 2000 et al.). A meta-analysis has shown an incidence rate of liver toxicity of 2.6% with isoniazid and rifampicin co administration, but only 1.1% with rifampicin alone and 1.6% with isoniazid alone.¹⁹ The conversion of monooacetyl hydrazine, a metabolite of INH to toxic metabolites via cytochrome P450 leads to hepatotoxicity. Patients on concurrent rifampicin therapy have an increased incidence of hepatitis, which has been postulated to be due to rifampicin-induced cytochrome P450 enzyme-induction, causing an increased production of toxic metabolites from acetyl hydrazine (Ellard, G.A. and P.T. Gammon, 1976 et al) (Wing-Wai, Y. et al). Rifampicin increases the metabolism of INH by acetylation and hydrolysis to isonicotinic acid and hydrazine, both of which are hepatotoxic on activation by cytochrome P450. The plasma half life of AcHz (metabolite of INH) is shortened by rifampicin. AcHz is quickly converted to its active metabolites i.e., reactive acylating species which bind covalently to the liver macromolecules causing hepatocyte injury. Thus there is a higher incidence of liver necrosis caused by INH and rifampicin in combination (Wing-Wai, Y. and L. Chi-Chiu, 2007 et al).

Since both CCl₄ and rifampicin-isoniazid combination involve activation by cytochrome P450, subsequent damage to the hepatocellular membrane by the toxic intermediate and increase in lipid peroxidation, the possible hepatoprotective mechanism of Acalypha indica would be inhibition of cytochrome P450 leading to inhibition of the lipid peroxidation, stabilization of the hepatocellular membrane and enhancement of protein synthesis (Zutshi et al and C.L. Chopra, 1987) (Wing-Wai, Y. and L. Chi-Chiu, 2007 et al). Have demonstrated that Piperine can enhance blood levels of drugs like vasicine, pterine, propanolol, theophylline, rifampicin when co administered with them. Piperine has been reported to enhance the bioavailability of a number of drugs by a non-specific and non-competitive inhibition of metabolic enzymes. It has been shown to inhibit both the monooxygenases and the conjugating (phase II) enzyme UDP-glucuronyl-transferase1. Thus the effect of potentiation of piperine may be attributed to the inhibition of metabolizing enzymes which metabolise the active constituent of Acalypha indica which is punarnavine, thus accounting for the increase in the hepatoprotective activity of Acalypha indica.

On the basis of the obtained results in this study it can be concluded that the roots of Acalypha indica exert a protective effect against CCl₄ and Rifampicin-Isoniazid induced hepatocellular damage. The flavonoids present in Acalypha indica may probably prevent the accumulation of excessive free radicals and protect the liver against CCl₄ and Rifampicin-Isoniazid intoxication. It was also found that piperine had a dose dependent potentiating effect on the hepatoprotective activity of Acalypha indica which can have important clinical implications in the future treatment of liver disorders.

**Table 1: Effect of acalypha indica extract ccl₄ induced Hepatotoxicity- Biochemical parameters**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>ALP</th>
<th>SGOT</th>
<th>SGPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control(a)</td>
<td>132.26+</td>
<td>3.013</td>
<td>134.26+</td>
</tr>
<tr>
<td>2</td>
<td>Toxic(b)</td>
<td>404.17+</td>
<td>6.427</td>
<td>4080.22+</td>
</tr>
<tr>
<td>3</td>
<td>Standard</td>
<td>223.60+</td>
<td>1.622</td>
<td>444.70+</td>
</tr>
<tr>
<td>4</td>
<td>Test Al 150mg/kg</td>
<td>232.59+</td>
<td>8.019</td>
<td>1850.36+</td>
</tr>
<tr>
<td>5</td>
<td>Test 300mg/kg</td>
<td>135.63+</td>
<td>2.15</td>
<td>148.56+</td>
</tr>
</tbody>
</table>

All Values are expressed as mean ± SEM. N=6 (a) as compared to control group (b) as compared toxic group. Analysis by one way ANNOVA followed by Dunnet’s test * significant as p<0.01.
Table 2: Effect of acalypha indica extract Rifampicin-isoniazid induced Hepatotoxicity

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>ALP</th>
<th>SGOT</th>
<th>SGPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>110.93+ 3.756</td>
<td>104.80+ 2.201</td>
<td>27.36+ 0.576</td>
</tr>
<tr>
<td>2</td>
<td>Toxic</td>
<td>267.26+ 1.042</td>
<td>203.73+ 6.33</td>
<td>44.46+ 0.46</td>
</tr>
<tr>
<td>3</td>
<td>Standard</td>
<td>137.40+ 3.63</td>
<td>133.36+ 1.47</td>
<td>32.33+ 1.369</td>
</tr>
<tr>
<td>4</td>
<td>Test-150mg/kg</td>
<td>211.40+ 6.65</td>
<td>180.20+ 0.664</td>
<td>42.26+ 0.2714</td>
</tr>
<tr>
<td>5</td>
<td>Test-300mg/kg</td>
<td>115.35+ 4.099</td>
<td>102.50+ 13.65</td>
<td>27.53+ 0.563</td>
</tr>
</tbody>
</table>

All values are expressed as Mean± SEM, N=6
Significant p<0.01

ACKNOWLEDGMENTS
The authors are thankful to CVM College of pharmacy, Karimnagar, India for their technical assistance.

REFERENCES