Hepatoprotective Efficacy of *Coleus amboinicus* Leaf Extract on Carbon Tetrachloride Induced Liver Damage in Rats.

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Abstract : The present investigation was aimed to study the protective role of ethanolic leaf extract of *Coleus amboinicus* against carbon tetrachloride induced hepatotoxicity in rats. The extract was administered at 200 mg/kg of body weight and the hepatoprotective activity was assessed by measuring biochemical parameters like ALT, AST, ALP, ACP, Total Protein and Total bilirubin. Biochemical studies showed that there was significant increase in the levels of ALT, AST, ALP, ACP and Total bilirubin while, the level of Total protein was decreased when rats were treated with carbon tetrachloride 2 ml/kg once a week for the period of 30 days. The altered level of these liver biomarkers was retrieved significantly near to control level when rats were given access to the ethanolic extract of *Coleus amboinicus* (EECE) at the dose of 200 mg/kg of body weight. The results of this study strongly indicate that EECA has got a potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats.

Keywords: Carbon Tetrachloride, *Coleus amboinicus*, ALT, AST, ALP, ACP, total bilirubin and total protein, Rat.

Introduction

The liver plays major and most essential roles in the human body which performs numerous vital functions such as cleansing, biotransformation, detoxification, synthesis and export of various plasma proteins, integrative role in the intermediary metabolism of carbohydrates, amino acids, and lipids (Goldman and Andrew, 2012). Therefore, liver disorders seriously and adversely affects the health and life expectancy. Exposure of toxic substances, alcohol consumption, metabolic ailment, pathogens and genetic alterations are some of main causes of most of the hepatic diseases (Achliya *et al.*, 2014). Changing lifestyle is also responsible for malfunctioning of liver and considered as a major health problem (Wolf, 1999). In addition constant use of synthetic drugs for treatment of liver disorders is inadequate and lead to different kind of side effects (Guntupalli, *et al.*, 2006). It has been realized that traditional herbal medicines prove to be safe and do not produce side effects. Hence, effective indigenous medicinal plants need to be explored and analyzed for their hepatoprotective nature. Importance of herbal drugs has gained popularity in recent years because of their safety, efficacy and cost effectiveness. A large number of plants and formulations have been widely monitoring for their hepatoprotective activity. This approach will help exploring the real beneficial value of these natural pharmaco-therapeutic agents and standardizing the dosage treatment on evidence-based findings to become more than a common trend (Agarwal, 2001). Herbs are also believed to have protective effects on high level exposure of free radicals that can cause the damage of cellular biochemistry. Free radicals can cause many diseases and can contribute to the ageing process (Anantha, *et al.*, 2012; Ames *et al.*, 1993). The harmful action of the free radicals however can be blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism. A number of medicinal plants have been used from time to time and to evaluate their antioxidant and hepatoprotective potentials such as *Solanum trilobatum* (Shahjahan *et al.*, 2004); *Cassia fistula* (Ilavarasan *et al.*, 2005); *Albizia lebbeck* (Resmi *et al.*, 2006); *Aegle marmelos* (Singanan, 2007); *Alafia multiflora* (Tsala *et al.*, 2010); *Solanum pubescens* (Pushpalatha and Ananthi, 2012); *Hedychium spicatum* (Thapliyal *et al.*, 2014), *Taraxacum officinale* (Sheikh *et al.*, 2015) and *Rosemarinus officinalis* (Akaram, *et al.*, 2016).

Carbon tetrachloride (CCl₄) is a potent hepatotoxin in experimental hapatopathy. The hepatotoxic effects of CCl₄ are mainly due to its active metabolite, trichloromethyl (CCl₃) (Johnson and Kroening, 1998 and Dial, 2010). Carbon tetrachloride is metabolized into trichloromethyl (CCl₃) radical due to the catalytic activity of CYP 450 2E enzyme and is then converted to trichloromethyl peroxy radical by superoxide anions and this trichloromethyl peroxy radical is the main culprit that leads to hepatotoxicity. This radical covalently bind to sulphhydryl group of membrane GSH, protein thiols and unsaturated lipids. This association of radicals with cellular macromolecules leads to lipid per oxidation (Lee *et al.*, 2004 and Wang *et al.*, 2014). One of the major defense mechanisms is involvement of antioxidant enzymes such as superoxide dismutase SOD, catalase (CAT), and Glutathione per oxidase (GPx), which converts active oxygen molecules into non toxic compounds. Scavenging of free radicals is one of most prominent anti oxidation mechanism that inhibits the chain reaction of lipid per oxidation. Carbon tetrachloride induces hepatotoxicity and decreases the level of antioxidant enzymes (Veenukumar and Latha, 2002). The rise in serum biomarkers i.e. Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Cholesterol has been reported due to damage in the liver. The level of these biomarkers was
reported to decrease due to the use of the methanolic extracts of *Casuarina equisetifolia*, *Cajanus cajan*, *Glycosmis pentaphylla*, *Bixa orellana*, *Argemone mexicana* (Ahsan et al., 2009).

Since, the leaves of *Coles amboinicus* were not evaluated for hepatoprotective efficacy, therefore, the present study was designed to evaluate the hepatoprotective effect of ethanolic leaf extract of *Coles amboinicus* (EECA) on ALT, AST, ALP, ACP, total protein and total bilirubin in rats exposed to carbon tetrachloride.

**Materials and Methods**

The leaves of *Coles amboinicus* were collected from local area of Bhopal India, in the months of February-March. The plant was identified and authenticated by H.O.D. Botany, Safia Science College, Bhopal, India. The voucher specimens bearing number 509/Bot/Safia/14 were submitted in the said department for future references.

**Preparation of the Plant Extracts**

The leaves of *Coles amboinicus* was dried in the dark and ground to a powder to obtain ethanolic extracts. Powder was subjected to Ethanol extraction in Soxhlet extractor using 90% ethanol three times and the solvent removed by vacuum distillation. Thus semi solid plant extract was kept frozen at 4°C and used when necessary for the enzymatic tests.

**Experimental Animals**

Twenty four healthy male albino wistar rats weighing 120-150g were procured from animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal Madhya Pradesh India, kept in cages at temperature 22 ± 2°C with 12 hours light and 12 hours dark cycle. All the animals were acclimatized for one week. The animals were given standard feed pellets and water *ad libitum*.

**Experimental design**

The animals were divided into four groups of six rats each. Group I – Control, received distilled water daily for 30 days and serves as normal control. Group II - received 2 ml/kg b. wt. CC1, weekly in olive oil (1:1, v/v, i. p.) for 30 days and served as toxicity control. Group III – received daily dose of 200 mg/kg b. wt. *Coles amboinicus* leaf extract for 30 days. Group IV - received 2 ml/kg b. wt. CC1, weekly in olive oil (1:1, v/v) along with daily dose of 200 mg/kg b. wt. *Coles amboinicus* leaf extract for 30 days.

**Biochemical Estimations**

The animals were euthanized with chloroform and dissected thereafter. The blood was collected by the heart puncture and allowed to clot for 30 minutes at room temperature. The collected blood was centrifuged at 3000 rpm for 15 minutes so as to get the serum. The serum was subjected for the estimation of levels of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP), total bilirubin (TB) and total protein (TP) using commercially available kits.

**Statistical Analysis**

Data were expressed in Mean SD. Statistical comparison between different groups were done by using One Way ANOVA followed by Benferroni’s test. P<0.05 and P<0.001 were considered as levels of significance.

**Results**

In control rats the level of ALT was 38.648±4.353 IU/L. However, it was significantly (p<0.001) elevated to 160.45±11.787 IU/L in CCl intoxicated group of rats. The level of ALT in the group of rats treated with 200 mg/kg b. wt. EECA was 36.668±5.77 IU/L, confirming the non-toxic nature of the EECA. However, a significant (p<0.001) decrease in the level of ALT to 83.248±6.888 IU/L was observed in the group IV of rats supplied with EECA at 200 mg/kg followed by CCl, intoxication in comparison to rats of group II (Table 1 & Fig.1-A).

A highly significant (p<0.001) elevation of AST to 281.195±10.595 IU/L was observed in the group of rats exposed to CCl, as compared to that of the control (109.446±8.209 IU/L). The group of rats supplied with only EECA at 200 mg/kg had the value of AST in close proximity to control viz. 122.195±9.791 IU/L. However, EECA at 200 mg/kg showed highly significant (p<0.001) protection in combating the damage in rats exposed to CCl, by decreasing its level to 161.383±17.689 IU/L (Table 1 & Fig.1-B).

The level of ALP in the control group of rats was 122.693±6.244 IU/L and exposure of rats to CCl, caused a highly significant (p<0.001) elevation in the level of ALP to 302.231±14.78 IU/L. The group of rats supplied with only EECA at 200 mg/kg showed the level of ALP as 139.296±12.549 IU/L. However, the rats inebriated with EECA at 200 mg/kg along with CCl, showed the protection of herbal extract by decreasing the level of ALP to 174.208±13.39 IU/L (p<0.001) (Table 1 & Fig.1-C).

In control group of rats, the level of ACP was 6.080±0.710 IU/L. However, the level of ACP was highly significantly (p<0.001) elevated to 17.22±1.235 IU/L in the group of rats intoxicated with CCl. But the EECA at 200 mg/kg for 30 days has not shown any side effects as the level of ACP exposure of rats to CCl caused a highly significant (p<0.001) elevation in the level of总蛋白 to 122.693±6.244 IU/L and exposure of rats to CCl, caused a highly significant (p<0.001) elevation in the level of ALP to 302.231±14.78 IU/L. The group of rats supplied with only EECA at 200 mg/kg showed the level of ALP as 139.296±12.549 IU/L. However, the rats inebriated with EECA at 200 mg/kg along with CCl, showed the protection of herbal extract by decreasing the level of ALP to 174.208±13.39 IU/L (p<0.001) (Table 1 & Fig.1-C).

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In control group of rats, the level of total protein (TP) was 11.057±0.804 g/dL and highly significant (p<0.001) decrease in the level of total protein to 6.348±0.738 g/dL was observed in the group of rats subjected to CCl, intoxication. The only herb treated group i.e. the group which received EECE at 200 mg/kg has not shown any...
significant variation in the level of total protein (9.646±0.837 g/dL). The treatment of the rats with 200 mg/kg of EECA alongside CCl₄ has shown the protection by highly significantly (p<0.001) increasing the level of total protein to 10.226±1.011 g/dL (Table 1 & Fig.1-E).

The level of total bilirubin (TB) in control group of rats was 0.418±0.053 mg/dL. However, CCl₄ exposure elevated the level to 2.037±0.392 mg/dL, (p<0.001). The group treated with EECA at 200 mg/kg showed the near normal levels of total bilirubin viz. 0.34±0.058 mg/dL. However, the rats supplied with EECA at 200 mg/kg, simultaneously subjected to CCl₄ intoxication has shown the highly significant (p<0.001) restoration by lowering the level of total bilirubin to 0.543±0.047 mg/dL (Table 1 & Fig.1-F).

Discussion
Liver damage was induced by CCl₄ in an animal model is used for the screening of hepatoprotective activity. Liver damage causes and releases the enzymes ALT, AST, ALP, ACP and total protein and total bilirubin in the serum due to the membrane and cellular damages of the liver. Number of medicinal plants has been screened for their therapeutic potential in reducing the liver ailments (Orhan, et al., 2007 and Paula Cordero-Pérez, et al., 2013).

In our study, the level of ALT in control group of rats was 38.648±4.353 IU/L, whereas, in CCl₄ group, the level was

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**Fig. 1** Showing the level of A –ALT; B –AST; C -ALP; D –ACP; E -TP and F -TB in rats treated with 200 mg/kg body weight ethanolic extract of *Coleus amboinicus* (EECA) leaves and intoxicated with 2 ml/kg b. wt. CCl₄ weekly in olive oil (1:1, v/v) for 30 days
explained that the serum levels of transaminases returned increased the alkaline phosphatase (ALP) activity and CCl . Amadi et al. (2010) reported that Gimelina arborea mg/kg of EECA and simultaneously exposed to 2 ml/kg of CCl treated group by 42.359% in the rats treated with 200 139.296±12.549 IU/L, which was well within the limits of those of other investigators (Fakurazi 2008).

Our results were in line with level was decreased by 42.608% in the group treated with EECA on AST is concerned, the level observed in the herb tissues and in liver (Dial, 2010). As far as the effect of Aspartate aminotransferase (AST) occurs in various animal, but is particularly concentrated in the liver, bile duct, kidney, bone and the placenta. The increase in activity is present in tissues throughout the entire body of the animal, but is particularly concentrated in the liver, CCl treated group of rats. Our results were in accordance with those of other researchers (Al-Qarawi, 2000). Kim and Wycoff (2011) recorded an increase in alanine aminotransferase activity in the rats treated with Gimelina arborea and reported that ALT also (Al-Qarawi, 2000). Kim and Wycoff (2011) observed a dose-dependent significant increase when treated with ethanol leaf-extracts of G. arborea (Offor, et al., 2015).

In the group of rats supplied with 200 mg/kg of EECA, the level of ALT was reduced by 73.343% when compared with only CCl4 (2 ml/kg, i.p.) treated group. The results of the present study contrast to that of only CCl (2 ml/kg, i.p.) treated group. Nevertheless, the level of total bilirubin in the group supplied with 200 mg/kg of EECA+ CCl, the level of ACP observed was 8.508±0.89* (-50.632%) in the 200 mg/kg EECA treated group was 9.646±0.83* (+34.190%) in contrast to that of only CCl (2 ml/kg, i.p.) treated group. The level of total bilirubin in the group supplied with 200 mg/kg of EECA was 9.646±0.837 g/dL. But the same was elevated by +37.922 % in the group treated with 200 mg/kg of EECA+ 2 ml/kg of CCl, in contrast to that of only CCl (2 ml/kg, i.p.) treated group. All data represented in Mean±SD, n=6, p<0.001 compared to CCl4 treated group. + = % increase and - = % decrease. CCl4 group was compared with control and the rest of groups were compared with CCl4 treated group.

Table: 1- Showing the effect of 200 mg/kg body weight ethanolic extract of Coleus amboinicus (EECA) leaves on ALT, AST, ALP, ACP, TP and TB in rats intoxicated with 2 ml/kg b. wt. CCl4 weekly in olive oil (1:1, v/v) for 30 days for 30 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT IU/L</th>
<th>AST IU/L</th>
<th>ALP IU/L</th>
<th>ACP IU/L</th>
<th>TP g/dL</th>
<th>TB mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.648±4.353</td>
<td>109.446±8.209</td>
<td>122.693±6.244</td>
<td>6.08±0.710</td>
<td>11.057±0.80</td>
<td>0.418±0.05</td>
</tr>
<tr>
<td>CCl4</td>
<td>160.45±11.78 (+75.912%)</td>
<td>281.195±10.59 (+61.078%)</td>
<td>302.231±14.78 (+59.404%)</td>
<td>17.22±1.23 (+64.692%)</td>
<td>6.348±0.73 (-42.588%)</td>
<td>2.037±0.39 (+79.479%)</td>
</tr>
<tr>
<td>EECA</td>
<td>36.668±5.77* (-77.146%)</td>
<td>122.195±9.79* (-56.544%)</td>
<td>139.296±12.54* (-50.462%)</td>
<td>8.501±0.86* (-47.688%)</td>
<td>9.646±0.83* (+34.190%)</td>
<td>0.34±0.05* (-83.308%)</td>
</tr>
<tr>
<td>EECA + CCl4</td>
<td>83.248±6.88* (-48.115%)</td>
<td>161.383±17.68* (-42.608%)</td>
<td>174.208±13.39* (-42.359%)</td>
<td>9.008±0.89* (-50.632%)</td>
<td>10.226±1.01* (+37.922%)</td>
<td>0.543±0.047* (-73.343%)</td>
</tr>
</tbody>
</table>

All data represented in Mean±SD, n=6, p<0.001 compared to CCl4 treated group. + = % increase and - = % decrease. CCl4 group was compared with control and the rest of groups were compared with CCl4 treated group.

very significantly elevated by +75.912%. When the rats were supplied with 200 mg/kg of ethanolic extract of Coleus amboinicus (EECA) for 30 days, the level of ALT observed was 36.668±5.77 IU/L which was almost similar to that of control group, thus showing the safety of the herbal extract at the selected dose. But, in the group of rats treated with the 200 mg/kg of EECA the level of ALT was reduced by 48.115%, thereby, proving the protective potential of the Coleus amboinicus leaves in improving the liver health. Similar trend was seen by other researchers also (Al-Qarawi, et al., 2000). Kim and Wycoff (2011) recorded an increase in alanine aminotransferase activity of rat treated with Gimelina arborea and reported that ALT is present in tissues throughout the entire body of the animal, but is particularly concentrated in the liver, bile duct, kidney, bone and the placenta. The increase in activity is probably because there was damage to the animals’ organs.

Aspartate aminotransferase (AST) occurs in various tissues, but remain in high concentrations in muscular tissues and in liver (Dial, 2010). As far as the effect of EECA on AST is concerned, the level observed in the herb treated group was 122.195±9.791 IU/L. Nevertheless, the level was decreased by 42.608% in the group treated with 200 mg/kg of EECA and 2 ml/kg of CCl, as against the only CCl treated group of rats. Our results were in line with those of other investigators (Fakurazi, et al., 2008).

Similarly, the level of ALP in group treated with EECA was 139.296±12.549 IU/L, which was well within the limits of normality. But, the level was declined in comparison to CCl, treated group by 42.359% in the rats treated with 200 mg/kg of EECA and simultaneously exposed to 2 ml/kg of CCl. Amadi et al. (2010) reported that Gimelina arborea increased the alkaline phosphatase (ALP) activity and explained that the serum levels of transaminases returned to normal with the healing of hepatic parenchyma and regeneration of hepatocytes. Our results were supported by the outcome of the findings of other investigators (Thangathirupathi, et al., 2013). Alkaline phosphatase (ALP) activity in the rats showed a dose-dependent significant increase when treated with ethanol leaf-extracts of G. arborea (Offor, et al., 2015).

In the group of rats supplied with 200 mg/kg of EECA, the level of ACP observed was 8.508±0.89* (-50.632%) in the 200 mg/kg EECA treated group was 9.646±0.837 g/dL. But the same was elevated by +37.922 % in the group treated with 200 mg/kg of EECA+ 2 ml/kg of CCl, in contrast to that of only CCl (2 ml/kg, i.p.) treated group. The level of total bilirubin in the group supplied with 200 mg/kg of EECA was 0.34±0.058 mg/dL, however, supplying the animals with 200 mg/kg of EECA alongside CCl reduced the level by 73.343% when compared with only CCl4 treated group. The results of the present study were in accordance with those of other researchers (Bhuvaneswari, et al., 2014).

It is quite evident from the results that the leaves of Coleus amboinicus do possess a very good hepatoprotective potential which is due to the presence of various phytochemicals like alkaloids, flavonoids, terpenoids, saponins, tannins, phenolic compounds and amino acids in its ethanolic extract. Recently we found that Coleus amboinicus leaves possess antioxidant properties (Aahirwar, et al., 2016).

The marked elevations in bilirubin level in the serum of CCl-intoxicated rats were significantly decreased in the EECA-treated animals. Bilirubin is the conventional indicator of liver diseases. These biochemical restorations
may be due to the inhibitory effects on cytochrome P450 or/and promotion of its glucuronidation. Decrease in total serum protein was observed in CCl4 treated rats that may be associated with the decrease in the number of hepatocytes, which in turn may result in the decreased hepatic capacity to synthesize protein. However the level of total protein restored to almost normal with the treatment of EECA.

Conclusion

Increased activity of serum ALT, AST, ALP, ACP in intoxicated rats, as evident in the present study, can be ascribed to the cellular damage of the liver because these are cytoplasmic in nature and are released into the circulation after cellular damage. Decrease in total serum protein was observed in CCl4 treated rats that may be associated with the decrease in the number of hepatocytes, which in turn may result in the decreased hepatic capacity to synthesize protein. Coleus amboinicus leaves offer protection to liver against adverse effects related to CCl4, by restoring elevated serum enzymes.

References


