ANTIOXIDANT AND HEPATOPROTective ACTIVITIES OF VERNONIA CINEREA EXTRACT AGAINST CCL4 INDUCED HEPATOMATOCity IN ALBINO RATS

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ABSTRACT
In the present study, we investigated the protective effect of *Vernonia cinerea* (F. Asteraceae) against carbon tetrachloride (CCL4) induced hepatotoxicity in albino rats. Hepatotoxicity was successfully induced by injecting CCL4 (0.5ml/kg body weight) intraperitoneally. Ethanol extract of *Vernonia cinerea* (250mg/kg) administrated intraperitoneally along with CCL4 for 7 days. The general observation, mortality, biomarker enzymes like lactate dehydrogenase (LDH), alkaline phosphatase (ALP), diagnostic enzyme markers like aspartate aminotransferase (AST), alanine aminotransferase (ALT) and antioxidants such as glutathione (GSH), Vit-C, glutathione peroxidase (Gpx), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) were monitored after 7 days of the last dose. CCL4 caused liver damage as evident by statistically significant (p<0.001) increased in plasma activities of AST, ALT, ALP, LDH. There were general statistically significant losses in activities of SOD, CAT, GPx, GSH, Vit-C, and an increase in MDA in the liver of CCL4-treated group compared with the control group. However, *Vernonia cinerea* were able to counteract these effects. The present results suggest that the plant can act as hepatoprotective against CCL4 toxicity and that the mechanism by which they exert hepatoprotection modulating antioxidant status.

Keywords: *Vernonia cinerea*, Antioxidant, Hepatotoxicity, Carbon tetrachloride, Lipid Peroxidation, Liver Marker Enzymes.

INTRODUCTION
Humans are continuously exposed to different kinds of chemicals such as food additives, industrial chemicals, pesticides and other undesirable contaminants in the air, food and soil. Most of these chemicals induce a free radical-mediated lipid peroxidation leading to disruption of biomembranes and dysfunction of cells and tissues. Therefore lipid peroxidation is a crucial step in the pathogenesis of free radical-related diseases including inflammatory injury and hepatic dysfunctions. It is also thought that antioxidants play a significant role in protecting living organism from the toxic effect of various chemicals by preventing free radical formation1.

The free radical-mediated hepatotoxicity can be effectively managed upon administration of such agents possessing antioxidants2, free radical scavengers3 and anti-lipid peroxidation4,5 activities. Apart from the natural antioxidant defense system, there are various synthetic antioxidants in use but these compounds have been reported to have various side effects. In this context natural compounds isolated from plants deserve significance.

Carbon tetrachloride (CCL4) is a potent hepatotoxic agent causing hepatic necrosis, and is widely used in animal models for induction of acute and chronic liver injury. It also induces hydropic degeneration, fatty changes, cirrhosis and hepatoma. CCl4-induced hepatotoxicity is believed to involve two phases. The initial phase involves the metabolism of CCl4 by cytochrome P450 to the trichloromethyl radicals (CCl3* and/or CCl3O), which lead to membrane lipid peroxidation and finally to cell necrosis6. The second phase of CCl4-induced hepatotoxicity involves the activation of Kupffer cells, which is accompanied by the production of proinflammatory mediators7.

It has been reported that treatment with CCl4 in mice and rats caused the release of AST and ALT, hepatocellular necrosis, decreased levels of antioxidative enzymes and increased lipid peroxidation products. The hepatotoxic effect induced by CCl4 could be reduced by treatment with dietary phytochemicals and antioxidants such as silymarin, flavonoids, curcumin, vitamin C and E8. In recent years, many researchers have become increasingly interested in herbal and edible plant extracts that possess hepatoprotective activities.

*Vernonia cinerea* (F. Asteraceae) is a common weed throughout India and it is well known as “Sahadevi” (Sanskrit), Naichette (or) Mukuthipundu. It has many therapeutic uses in different traditional medicine of the world. Different parts of the plant are of different therapeutic values. To mention a few, the plant is used for malaria fever, worms, pain, inflammation, infections, diuresis, cancer, abortion, and various gastro-intestinal disorders9. Other Vernonia species that shared some of these therapeutic values include: *Vernonia brachycalyx*, *Vernonia brasiliiana*, *Vernonia herbacea*, *Vernonia subligera* and *Vernonia coloralia*.

In traditional system of medicine the whole plant with its small flowers is used medicinally to promote perspiration in febrile conditions. Co-administered with quinine it is
beneficial in malarial fevers. Poultice of the leaves is useful against guinea worms. Flowers are administered for conjunctivitis. The flower extract of the plant was used in adjuvant-induced arthritis. The present study has been designed to evaluate the hepatoprotective effect of Vernonia cinerea in CCl4 induced albino rats.

MATERIALS AND METHODS

Plant

The leaves of Vernonia cinerea were collected from Trivandrum, Kerala, India, during December 2009. The plant was authenticated by Botanical Survey of India, Coimbatore, India. The leaves were shade dried at room temperature and subjected to size reduction to a coarse powder by using dry grinder and passed through a sieve. It was extracted to exhaustion with ethanol using a shaker. The extract thus obtained was dried using a rotary evaporator under reduced pressure at 40°C.

Animal

The female wistar strains albino rats weighing between 150-180gm were obtained from KMCH Pharmacy College, Coimbatore. Animals were kept in animal house at an ambient temperature of 25°C and 45-55% relative humidity, with 12h each of dark and light cycles. They were fed with standard rodent pellet, trade name “Gold Mohur rat feed” (Hindustan lever Ltd, India) and tap water ad libitum. The study was conducted after obtaining clearance from Institutional animal ethical committee (Bio.Chem/3/2005).

Chemicals

All the chemicals were obtained from Loba chemie Pvt. Ltd, Mumbai and S.D. Fine Chemicals, Chennai.

Induction of experimental hepatotoxicity

The liver injury was induced by CCl4 according to methods described previously10, 11. Liver damage was induced in rats with a 1:1 (v:v) mixture of CCl4 and olive oil, administered intraperitoneally at a dose of 0.5 ml/kg body weight for 7 days.

Experimental set up

The rats were randomly divided into four groups of six animals in each.

Group I: Served as control

Group II: Administered CCl4 (0.5ml/kg/bw) for 7 days.

Group III: Administered CCl4 (0.5ml/kg/bw) + Silymarin for 7 days.

Group IV: Received simultaneously both Vernonia cinerea (250mg/kg/bw) and CCl4 (0.5ml/kg/bw)7 days.

At the end of the experiment period, the animals in different groups were sacrificed by cervical decapitation. Blood and tissue were collected for various examinations.

In vitro Antioxidant Assay

DPPH radical scavenging activity

The free radical scavenging activity was determined by the method of Shimada et al. (1992) and Yang et al. (2006)12, 13. The ethanolic extracts were dissolved with ethanol to prepare various sample solutions at 10, 20, 30, 40, 50µg/ml. Each extract solution (2ml) was mixed with 1ml of methanolic solution containing DPPH radicals, with a final concentration of 0.2mM DPPH. The mixture was shaken vigorously and maintained for 30min in dark. The absorbance was measured at 517nm. The absorbance of the control was obtained by replacing the sample with methanol. Quercetin was used as standard reference. The scavenging activity was calculated using the formula, Scavenging activity (%) = [(A517 of control-A517 of sample) / A517 of control] X 100.

ABTS Radical scavenging activity

The ABTS radical scavenging activity of the extract was measured by Rice-Evans et al., (1997)14. Monocation radical ABTS** (2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulphate and the mixture was allowed to stand in dark at room temperature for 12-16 hr before use. Different concentrations (10-50 µg/ml) of extract or standard (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with methanol to make 1 ml. The absorbance was read at 745nm and the % inhibition was calculated. The experiment was performed in triplicate.

Biochemical parameters

The following parameters were analysed to evaluate the hepatoprotective of Vernonia cinerea by the methods given below: Serum AST15, ALT16, ALP17, and LDH18, a liver homogenate was used for the analysis of Superoxide dismutase19, Catalase20, glutathione peroxidase21, Total reduced glutathione22, ascorbic acid23.

Determination of lipid peroxidation

MDA in liver homogenate and microsomal fraction was determined by the reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation24.

Statistical analysis

All data were expressed as the means ± standard deviation (SD). Student’s t- test was used to arrive at the statistically significant changes associated with various treatments. p<0.05 was regarded as significant.

RESULTS AND DISCUSSION

The liver is the major organ responsible for the metabolism of drugs and toxic chemicals, and therefore is the primary target organ for nearly all toxic chemicals25. The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years by using acute poisoning with CCl426. CCl4 is an extensively studied liver toxicant, and its metabolites such as trichloromethyl...
peroxy radical (CCl4 O₂) are known to be involved in the pathogenesis of liver damage.

In vitro antioxidant capacity of Vernonia cinerea extract, as assessed by DPPH & ABTS assay showed a dose-dependent activity. Which conform that the extract to possess antioxidant like properties, which may be a contributing factor for mitigation of CCl4 induced hepatotoxicity (Table 1).

Following CCl4 administration the level of all marker enzymes increased significantly in group II animals (p<0.001), as compared to normal controls (Table 2). Vernonia cinerea treatment caused a significant decreases (p<0.001) in the activities of all these enzymes in group IV animals. The increased activities of liver marker enzymes such as AST, ALT, ALP and LDH in the serum of CCl4 induced animals indicate damage to hepatic cells.

Lipid peroxidation has been implicated in the pathogenesis of hepatic injury by compounds like CCl4 and is responsible for cell membrane alterations. In the present study, significantly elevated level of lipid peroxides (p<0.001) observed in CCl4 administered rats (group II) indicated excessive formation of free radicals and activation of lipid peroxides system resulting in hepatic damage. The significant decline in the lipid peroxides content in the liver tissue of group IV rats indicated antilipid peroxidative effect of Vernonia cinerea (Table 3).

### Table 1: Effect of Vernonia cinerea (VC) (% inhibition) on DPPH and ABTS radical scavenging activity

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Quercetin</th>
<th>DPPH</th>
<th>ABTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>55.13±0.146</td>
<td>60.60±0.505</td>
<td>62.57±2.388</td>
</tr>
<tr>
<td>20</td>
<td>79.48±0.072</td>
<td>78.45±0.304</td>
<td>68.15±1.690</td>
</tr>
<tr>
<td>30</td>
<td>81.02±0.083</td>
<td>79.73±0.139</td>
<td>76.57±2.526</td>
</tr>
<tr>
<td>40</td>
<td>88.95±0.066</td>
<td>89.68±0.219</td>
<td>80.17±1.356</td>
</tr>
<tr>
<td>50</td>
<td>94.30±0.272</td>
<td>91.00±0.064</td>
<td>89.79±2.255</td>
</tr>
<tr>
<td>Ec-50(µg/ml)</td>
<td>20.00</td>
<td>22.00</td>
<td>12.00</td>
</tr>
</tbody>
</table>

Each value was expressed as the mean ± SD. (n=3)

*p=0.001 as compared with normal group; p<0.001 as compared with CCl4 induced group

### Table 2: Effect of Vernonia cinerea (VC) on liver marker enzymes in the serum of control and experimental animals.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (group I)</th>
<th>CCl4 induced (group II)</th>
<th>CCl4+silymarin (group III)</th>
<th>CCl4+VC (group IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/dl)</td>
<td>30.99±0.37</td>
<td>174.41±12.63</td>
<td>55.52±3.47</td>
<td>76.02±3.74</td>
</tr>
<tr>
<td>AST (IU/dl)</td>
<td>118.39±5.98</td>
<td>568.72±5.24</td>
<td>347.88±7.81</td>
<td>364.84±4.65</td>
</tr>
<tr>
<td>ALP (IU/dl)</td>
<td>117.39±2.10</td>
<td>343.44±7.56</td>
<td>211.29±11.22</td>
<td>250.55±11.48</td>
</tr>
<tr>
<td>LDH (IU/dl)</td>
<td>101.07±5.92</td>
<td>153.25±27.01*</td>
<td>117.19±7.22</td>
<td>127.19±12.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM for 6 animals in each observation

*p=0.001 as compared with normal group; p=0.0001 as compared with CCl4 induced group

### Table 3: Effect of Vernonia cinerea (VC) on the antioxidant status of liver in the control and experimental animals.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal (group I)</th>
<th>CCl4 induced (group II)</th>
<th>CCl4+silymarin (group III)</th>
<th>CCl4+VC (group IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (Unit/mg protein)</td>
<td>2.04±0.05</td>
<td>1.75±0.05*</td>
<td>2.02±0.08</td>
<td>1.99±0.13</td>
</tr>
<tr>
<td>CAT (IU/mg protein)</td>
<td>205.89±0.737</td>
<td>131.55±2.128*</td>
<td>203.070±1.317</td>
<td>205.050±0.827</td>
</tr>
<tr>
<td>GPx (µmol/min/mg protein)</td>
<td>11.37±0.452</td>
<td>5.183±0.087*</td>
<td>11.083±0.256</td>
<td>11.251±0.008</td>
</tr>
<tr>
<td>GSH (µg/mg protein)</td>
<td>49.83±0.229</td>
<td>27.262±0.311*</td>
<td>49.758±1.317</td>
<td>47.949±0.784</td>
</tr>
<tr>
<td>Vit-C (µg/mg protein)</td>
<td>39.83±0.119</td>
<td>37.610±0.400*</td>
<td>39.413±0.292</td>
<td>38.993±0.156</td>
</tr>
<tr>
<td>LP (µmol/mg protein)</td>
<td>0.216±0.001</td>
<td>0.263±0.000*</td>
<td>0.213±0.003</td>
<td>0.217±0.003</td>
</tr>
</tbody>
</table>

Values are mean±SEM for 6 animals in each observation

*p=0.001 as compared with normal group; p>0.001 as compared with CCl4 induced group

Values are expressed as SOD, superoxide dismutase (unit/min/mg protein); CAT, catalase (µmol/min/mg protein); GPx, glutathione peroxidase (µg of glutathione consumed/min/mg of protein); GSH, glutathione (µg/mg protein); Vit-C, Vitamin-C (µg/mg protein); LP, lipid peroxidation (µmoles of malonaldehyde (MDA) formed/mg protein/h).
SOD, CAT, GSH, GPx and Vit-C constitute a mutually supportive team of defence against reactive oxygen species (ROS). SOD (metalloprotein) is the first enzyme involved in the antioxidant defense by lowering the steady state of O$_2^-$. CAT is a hemoprotein, localized in the peroxisomes and catalyses the decomposition of H$_2$O$_2$ to water and oxygen. GPx, a selenoenzyme, present predominantly in liver and catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide.

### CONCLUSION

In the present study pretreatment with Vernonia cinerea (group IV) showed increased activity of antioxidant enzymes compared to CCl4 treated animals indicating the potentiality of Vernonia cinerea to act as an antioxidant by preventing the peroxidative damage caused by CCl4.

### REFERENCES


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**About Corresponding Author: Mr. G. Leelaprakash**

Mr. G. Leelaprakash working as a Sr. Lecturer in the PG Department of Biochemistry, Administrative Management College, Bangalore. He is having 9 years of teaching experience in PG Department. He completed Master Degree in University of Madras and Master of Philosophy in Bharathidasan University, Tiruchirappalli. Now he is pursuing Doctor of Philosophy in Biochemistry, Bharathiar University, Coimbatore. His Research area is "Role of Medicinal plants in Cancer Biology".