Comparative Effect of *Terminalia bellerica* Fruit Extract and its Active Principle Against Carbon Tetrachloride-Induced Toxicity in Rats.

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The protective effect of *Terminalia bellerica* fruit extract and its active principle (gallic acid: 3,4,5-trihydroxybenzoic acid) were investigated against carbon tetrachloride induced toxicity in rats. Carbon tetrachloride caused significant increase in the activity of alkaline phosphatase, transaminases and protein content. Hepatic lipid peroxidation increased significantly while reduced glutathione level in liver decreased after toxicant administration. Considerable inhibition was observed in glycogen content as well as in the activity of alkaline phosphatase, succinic dehydrogenase and adenosine triphosphatase in liver and kidney. Activity of acid phosphatase elevated significantly in both the organs. Recoupmnt was seen in almost all the parameters by therapy with extract and active principle intoxicant induced subjects. The degree of protection conferred by active principle was more as compared to ethanolic extract of *Terminalia bellerica*.

*Terminalia bellerica* Roxb. (TB, Combretaceae, 'bahera') is distributed throughout the forests of India. Among the various medicinal properties attributed to its significance, one is its therapeutic value in the treatment of liver disorders and indigestion*. The fruits of TB are reported to have purgative*, cardiac depressant and choleric effects*. It is one of the ingredients of ayurvedic purgative medicament *triphala*. Gallic acid (GA: 3,4,5-trihydroxybenzoic acid) is an active principle of *Terminalia bellerica* fruit. Therefore, present investigation aims to evaluate the protective potential of *Terminalia bellerica* fruit extract and its active principle against carbon tetrachloride (CCl₄) intoxication.

MATERIALS AND METHODS
Preparation of the extract:
Fruits of *Terminalia bellerica* were procured from authenticated Ayurvedic dealer and were identified by the taxonomist of Botany department of Jiwaji University, Gwalior. A voucher specimen (No. 336) has been deposited in Herbarium (Acronym JUG) of Jiwaji University, Gwalior. Fruits were dried, chopped and ethanolic extract was prepared (17.6% w/v). An aqueous suspension of crude extract in 2% gum acacia was administered to the animals orally. Gallic acid was procured from Sigma-Aldrich (Bangalore) and CCl₄ was procured from Ranbaxy (New Delhi). The other chemicals used in the study were procured from Sigma-Aldrich (Bangalore) and E-Merck (Germany). Toxicity was induced by 1.5 ml/kg (ip) CCl₄ mixed with liquid paraffin. Equal amount of liquid paraffin was given as vehicle.

Female rats of Sprague Dawley strain (130±10 g) were used for hepatoprotective studies. Animals were housed under standard conditions (25±2°C, 60-70% relative humidity and 14 h light and 10 h dark). The rats were fed on standard pellet diet (Hindustan Liver Ltd., New Delhi) and water *ad libitum*. Animals used in this study were treated and handled in accordance with the guidelines recommended by the Control and Supervision of Experiments on Animals (CPSEA), Chennai. Experimental protocol for treating animals was approved by IAEC (Institutional Animals Ethics Committee).
TABLE 1: EFFECT OF TREATMENTS ON THE ACTIVITY OF SERUM TRANSAMINASES.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>65.5±3.36</td>
<td>47.4±2.51</td>
</tr>
<tr>
<td>CCl₄</td>
<td>228.4±18.6*</td>
<td>436.0±22.0*</td>
</tr>
<tr>
<td>CCl₄+Extract</td>
<td>168.0±9.77**</td>
<td>247.0±17.0**</td>
</tr>
<tr>
<td>CCl₄+AP</td>
<td>109.0±9.39**</td>
<td>126.0±6.95**</td>
</tr>
<tr>
<td>CCl₄+Sily</td>
<td>79.8±6.28**</td>
<td>117.0±10.8**</td>
</tr>
<tr>
<td>One-way ANOVA df</td>
<td>6.20</td>
<td>6.20</td>
</tr>
<tr>
<td>F</td>
<td>49.03</td>
<td>153.87</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

AP=active principle, Sily=silymarin. Values are mean±SEM, n=5, * P<0.05, when compared with control, ** P<0.05, when compared with CCl₄-treated group.

Treatments:
Animals were divided into five groups of five animals each. Group 1 served as normal control. Other four groups were administered CCl₄ (1.5 ml/kg, ip). Group 2 was treated as experimental control. Groups 3-4 were administered Terminalia bellerica extract (400 mg/kg, po) and active principle (200 mg/kg, po) respectively after 24 h of CCl₄ administration. Group 5 received a reference drug silymarin (50 mg/kg, po) and served as positive control. All the animals were sacrificed after 24 h of last treatment.

Just before the necropsy, blood was collected by puncturing the retro-orbital sinus, serum was separated at 2000 rpm. and various parameters viz - alkaline phosphatase, protein, and transaminases were processed. Immediately after necropsy, liver and kidney were removed. Fresh tissues were processed for the estimation of glycogen. The quantitative measurement of lipid peroxidation was done by measuring the concentration of thiobarbituric acid reactive species (TBARS) in liver. Reduced glutathione was estimated in the liver homogenate using dithionitrobenzoic acid (DTNB). Tissue homogenates were prepared in ice-cold hypotonic solution for the estimation of alkaline and acid phosphatase, adenosine triphosphatase and succinic dehydrogenase. Data were expressed as means±SEM. and were statistically assessed by one-way analysis of variance (ANOVA). Difference between, animals of treated and control groups were calculated by Student’s 't' test. P<0.05 was taken as significant.

RESULTS

Tables 1 and 2 demonstrate that administration of CCl₄ caused significant increase in the activity of transaminases, alkaline phosphatase and protein content in experimental control group. Because CCl₄ is associated with a variety of biochemical abnormalities and these are attributed to the release intracellular constituents into the circulation. Thus elevated level of these enzymatic variables clearly indicated the cellular leakage and loss of the functional integrity of the cell membranes in liver. With the treatment of active principle significant recoupment was observed when compared with the crude extract of Terminalia bellerica. CCl₄ caused decrease in the activity of alkaline phosphatase whereas acid phosphatase activity increased significantly in liver and kidney. Active principle was found to be significantly effective in both the organs (Table 3). Appreciable fall was observed in glycogen content, activity of adenosine triphosphatase and succinic dehydrogenase after CCl₄ administration. Extract caused a marked reversal in the inhibitory effect of the enzymatic variables while active principle was found to be more effective and the values were very near to control in both the organs (Table 4). Toxicant exposure caused significant inhibition in the glycogen content in liver and kidney (P<0.05). These parameters were significantly recovered by the therapy of active principle but extract therapy was not effective at this
TABLE 3: EFFECT OF TREATMENTS ON ACID AND ALKALINE PHOSPHATASE.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Acid phosphatase (mg Pi/100 g/h)</th>
<th></th>
<th>Alkaline phosphatase (mg Pi/100 g/h)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Normal Control</td>
<td>234.6±16.3</td>
<td>267.6±18.3</td>
<td>76.2±5.42</td>
<td>2680±261</td>
</tr>
<tr>
<td>CCl₄</td>
<td>389.0±28.6*</td>
<td>369.6±19.0*</td>
<td>43.6±3.83*</td>
<td>1289±115*</td>
</tr>
<tr>
<td>CCl₄+Extract</td>
<td>277.0±14.7**</td>
<td>299.0±22.5**</td>
<td>55.6±4.19</td>
<td>1666±102**</td>
</tr>
<tr>
<td>CCl₄+AP</td>
<td>258.0±23.9**</td>
<td>292.0±21.3</td>
<td>60.0±4.00**</td>
<td>2209±131**</td>
</tr>
<tr>
<td>CCl₄+Sily</td>
<td>260.0±24.3**</td>
<td>271.0±23.0**</td>
<td>73.0±6.02**</td>
<td>2198±184**</td>
</tr>
<tr>
<td>One-way ANOVA df</td>
<td>6.20</td>
<td>6.20</td>
<td>6.20</td>
<td>6.20</td>
</tr>
<tr>
<td>F</td>
<td>9.33</td>
<td>4.84</td>
<td>9.79</td>
<td>12.61</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

AP=active principle, Sily=silymarin. Values are mean±SEM, n=5, *P<0.05, when compared with control, **P<0.05, when compared with CCl₄-treated group.

level (Table 5). There was a significant increase in lipid peroxidation on the contrary decrease was observed in the glutathione content after CCl₄ administration. Active principle and extract were effective in recouping these variables significantly (Table 6). The protective effects of these therapeutic agents were also compared with silymarin treated animals, which is used as a reference drug.

DISCUSSION

The results of the present study clearly demonstrate that the various biochemical alterations produced by CCl₄ in the serum and tissue were reversed significantly by the administration of the extract and active principle of Terminalia belerica. Results reveal that the administration of CCl₄ caused significant increase in the level of serum proteins, activity of serum alkaline phosphatase and

TABLE 4: EFFECT OF TREATMENTS ON ADENOSINE TRIPHOSPHATASE AND SUCCINIC DEHYDROGENASE

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Adenosine triphosphatase (mg Pi/100 g/h)</th>
<th></th>
<th>Succinic dehydrogenase (n mol KₙFe(CN)₆/min/mg protein)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Normal Control</td>
<td>2008±138</td>
<td>2480±130</td>
<td>45.2±2.96</td>
<td>39.0±2.82</td>
</tr>
<tr>
<td>CCl₄</td>
<td>997±57.0*</td>
<td>1033±61.4*</td>
<td>28.0±2.00*</td>
<td>26.8±2.67*</td>
</tr>
<tr>
<td>CCl₄+Extract</td>
<td>1685±97.4**</td>
<td>1649±96.3**</td>
<td>35.8±3.36</td>
<td>35.0±2.44</td>
</tr>
<tr>
<td>CCl₄+AP</td>
<td>1947±100**</td>
<td>1809±120**</td>
<td>40.6±2.25**</td>
<td>37.8±2.65**</td>
</tr>
<tr>
<td>CCl₄+Sily</td>
<td>2099±127**</td>
<td>2200±121**</td>
<td>44.0±2.57**</td>
<td>38.8±3.02**</td>
</tr>
<tr>
<td>One-way ANOVA df</td>
<td>6.20</td>
<td>6.20</td>
<td>6.20</td>
<td>6.20</td>
</tr>
<tr>
<td>F</td>
<td>21.41</td>
<td>32.32</td>
<td>8.59</td>
<td>4.36</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

AP=active principle, Sily=silymarin, values are mean±SEM, n=5, *P<0.05, when compared with control, **P<0.05, when compared with CCl₄-treated group.
TABLE 5: EFFECT OF TREATMENTS ON GYOCOGEN CONTENT

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Liver glycogen (mg/100 g)</th>
<th>Kidney glycogen (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>2801±135</td>
<td>86.4±4.95</td>
</tr>
<tr>
<td>CCl₄</td>
<td>1548±120*</td>
<td>58.0±3.12*</td>
</tr>
<tr>
<td>CCl₄+Extract</td>
<td>1919±121</td>
<td>72.0±3.65**</td>
</tr>
<tr>
<td>CCl₄+AP</td>
<td>2203±155**</td>
<td>79.0±5.91**</td>
</tr>
<tr>
<td>CCl₄+Sily</td>
<td>2435±165**</td>
<td>76.2±4.70**</td>
</tr>
<tr>
<td>One-way ANOVA df</td>
<td>6.20</td>
<td>6.20</td>
</tr>
<tr>
<td>F</td>
<td>14.49</td>
<td>6.60</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

AP=active principle, Sily=silymarin, values are mean±SEM, n=5, *P≤0.05, when compared with control, **P≤0.05, when compared with CCl₄-treated group.

Due to liver injury, the transport function of the hepatocytes gets disturbed, resulting in the leakage of serum alkaline phosphatase in plasma membrane thereby causing an elevation\textsuperscript{18}. Damage to the structural integrity of liver is reflected by an increase in the level of serum transaminases because these are cytoplasmic in location and release into circulation after cellular damage. It is generally accepted that the toxicity of CCl₄ depends on the cleavage of the carbon-chlorine bond to generate trichloromethyl and trichloromethyl peroxy radicals, which may contribute to the hepatotoxicity and subsequent increase in hepatic enzymes\textsuperscript{29,27}. In the present study author has also observed a rise in the level of AST and ALT in CCl₄-treated rats. The recoupment seen with gallic acid may be due to the substantial antioxidant properties. It may combine with reactive metabolites and lead to inactivate them, which may suppress the intracellular concentration of free radicals produced by CCl₄. Thus it may prevent the acute organ dysfunction and cellular injury thereby inhibiting the rapid leakage of these enzymes. Recoupment with the administration of different plant preparations are reported, e.g. Schisandra chinensis\textsuperscript{22}, Capris spinosa\textsuperscript{23}, Withania somnifera\textsuperscript{24}, Andrographis paniculata\textsuperscript{25}, Emblica officinalis\textsuperscript{26}, Mallotus japonicus\textsuperscript{27}.

It is also observed that CCl₄ caused significant decrease in the glycogen content of liver and kidney. The reason may be the disruption of glycogen storage, which is associated with dysfunctional and dystrophic changes in the liver and kidney due to inhibition of key enzymes in carbohydrate metabolism. CCl₄ brings about a rise in cytosolic free calcium, which may lead to glycogen mobilization, thus causing depletion in hepatic glycogen content\textsuperscript{28}. It may be assumed that active principle being an antioxidant reduces the stress to a considerable extent thereby reducing the demand for the excess sugar and recouping the glycogen content.

CCl₄ also caused significant increase in the activity of acid phosphatases. It may be due to the lysosomal imbalance resulting in destruction of the intact membranes. Administration of CCl₄ led to the assimilation of fat in the liver and kidney and demonstrates continuous process of autophagy and thus increases the activity of acid phosphatase\textsuperscript{29}. Extract may possess anti-inflammatory and lysosomal stability properties and obstructs the rise in the enzymatic activity. The increase in the activity of the lysosomal enzymes, in the organs after treatment with the CCl₄ suggested increased tissue catabolism and autophagy, which are possible sequences leading to renal damage\textsuperscript{30}. Alkaline phosphatase has been reported to be involved in the transport of metabolites across the cell membranes, synthesis of protein and certain enzymes, secretary activities and glycogen metabolism. Thus the alterations in

TABLE 6: EFFECT OF TREATMENTS ON HEPATIC LIPID PEROXIDATION AND REDUCED GLUTATHIONE

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Hepatic lipid peroxidation (n mol of MDA/mg protein)</th>
<th>Hepatic reduced glutathione (µ mol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.27±0.02</td>
<td>8.63±0.48</td>
</tr>
<tr>
<td>CCl₄</td>
<td>1.60±0.15*</td>
<td>3.70±0.20*</td>
</tr>
<tr>
<td>CCl₄+Extract</td>
<td>1.03±0.08**</td>
<td>5.20±0.31**</td>
</tr>
<tr>
<td>CCl₄+AP</td>
<td>0.58±0.04**</td>
<td>6.30±0.52**</td>
</tr>
<tr>
<td>CCl₄+Sily</td>
<td>0.35±0.03**</td>
<td>7.00±0.62**</td>
</tr>
<tr>
<td>One-way ANOVA df</td>
<td>6.20</td>
<td>6.20</td>
</tr>
<tr>
<td>F</td>
<td>57.10</td>
<td>20.86</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

AP=active principle, Sily=silymarin, values are mean±SEM, n=5, *P≤0.05, when compared with control, **P≤0.05, when compared with CCl₄-treated group.
the enzymatic activity may be due to the disturbance in the
secretory activity or in the transport of metabolites or may
be due to altered synthesis of certain enzymes.

Adenosine triphosphatase is lipid dependent
membrane bound enzyme any alteration in membrane lipid
leads to change in membrane fluidity, which in turn alters the
adenosine triphosphatase, mediated cellular functions.
In the present investigation, significant fall was observed in
the enzyme activity on CCl₄ exposure. This inhibition of
adenosine triphosphatase by CCl₄ exposure has also been
confirmed in other studies⁵⁸,₃¹. Therapy of active principle
showed significant improvement in the activity of enzyme.
Succinic dehydrogenase is a mitochondrial enzyme tightly
bound to the inner mitochondrial membrane. It plays an
important role in energy conversion. A significant fall in the
succinic dehydrogenase activity could result in serious
impairment of mitochondrial function and metabolic turnover.
This may be due to the structural and functional
organization of the mitochondrial activity. Active principle
may possibly play a role in retaining the impairment of
mitochondrial function.

Considerable lowering of reduced glutathione level was
observed in the liver, on the other hand, there was
remarkable increase in the activity of lipid peroxidation.
These studies are supported by various authors with the
administration of carbon tetrachloride⁵²-⁵⁵ increased TBARS
of liver indicated enhanced lipid peroxidation due to tissue
injury and failure of the antioxidant defense mechanism,
which prevents the formation of excess free radicals. As
active principle has antioxidant property, it may prevent free
radical generation thereby reducing oxidative stress.
Administration of active principle may promote the
conversion of GSSG into GSH by reactivation of hepatic
GSSG reductase enzyme in CCl₄ treated animals. The
availability of a sufficient amount of GSH thus increased
the detoxification of active metabolites of CCl₄. Here the
role of TB extract and its active principle in reversing the
elimination of hydrogen peroxide free radicals may be
visualized as a form of adaptation on the part of GSH
dependent defense system against lipid peroxidation. It also
appears that certain constituents of Andrographis
paniculata prevented liver membrane from undergoing lipid
peroxidation and showed improvement in the reduced
glutathione level⁵⁵.

Thus in the case of beneficial effect of the therapeutic
agents three types of possible interventions are expected.
(I) The chemical ingredients in the extracts have membrane
stabilizing effect. (II) Gallic acid may possess the ability to
block the bioactivation of CCl₄ by inhibiting P₄₅₀E₁ activity
and its expression. (III) Due to the presence of -OH and
-COOH groups GA may directly combine with free radicals
(·CCl₃) and form free radical adduct. Thus it may be
concluded that extract and active principle possess
protective potential but effect of active principle was more
pronounced in ameliorating CCl₄-induced toxicity.

ACKNOWLEDGEMENTS

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