Protective Effect of Ethanolic Extract of *Tamarindus indica* Against CCl₄ Induced Liver Damage in Rats

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**Abstract:** The aim of this study is to investigate the effect the fruit pulp ethanolic extract of *Tamarindus indica* in protecting liver damage induced by CCl₄. Thirty Wistar rats were used and divided into 5 groups of 6 rats each, the first group served as control and was given orally vehicle saline at 1ml/kg/day, the second group acts as CCl₄ control and received CCl₄ to induced damage to the liver, the third group treated with ethanolic extract of *Tamarindus indica* at 150 mg/kg/day and CCl₄, the fourth group was treated with silymarin as standard drug and CCl₄ and the fifth group was treated with 150 mg/kg/day ethanolic extract of *Tamarindus indica* alone, the experiment continued for 5 days, liver enzymes, AST, ALT and ALP were recorded as well as total bilirubin, total protein and albumin, heamatological parameters include RBCs, Hb, PCV, MCV and MCHC were measured. We found that, the ethanolic extract of *T. indica* ameliorated the damage caused by CCl₄ by lowering the levels of AST, ALT, ALP and the concentration of bilirubin and this effect was verified by improvement of histopathological picture in the livers of the group treated by the plant compared with the liver of CCl₄ group which exhibited severe centrilobular necrosis and fatty vacuolation. The study indicates that, *Tamarindus indica* ethanolic extract possess hepatoprotective ingredients.

**Key words:** *Tamarindus indica*, Protective activity, Carbon tetrachloride, Rats

**INTRODUCTION**

The liver is a vital and key organ involved in the metabolic functions and detoxification, so it is continuously exposed to xenobiotics; on the other hand drugs used in treatment of liver diseases are inadequate and possess adverse side effects. Therefore it is necessary to search for alternatives for the treatment of liver diseases in order to replace currently used drugs of doubtful efficacy and safety (Ozbek *et al*., 2004).

*Tamarindus indica* belonging to the family Caesalpinaceae and is widely used in folkloric medicine in Sudan and Known locally as Aradaib. *T. indica* fruits were used to treat fever, post partum remedy and measles (Roosita katrin *et al*., 2008).

Ethanolic extract of *T. indica* possess strong *in vitro* antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* *Salmonella paratyphi* *A* and *Pseudomonas aeruginosa* (Abdel Gadir *et al*., 2007, Daniyan and Muhammad 2008; Melendes and Capriles 2006).

The study of Ushanandini *et al*., (2006), clearly indicates that *T. indica* seed extract contains metabolites that are potent inhibitors of hydrolytic enzymes and toxic components of *Vipera russelle* venom. Havinga *et al*., (2010), reported that, one of the main applications of *Tamarindus* fruits is its use as a laxative.

In South coast community in Kenya, Nuguta *et al*., (2010), reported the use of *T. indica* as antimalarial. Maiti *et al*., (2004), found that, the aqueous extract of seed of *Tamarindus indica* was found to have potent antidiabetogenic activity that reduces blood sugar level in Streptozotocin (STZ) – induced diabetic male rat.

**MATERIALS AND METHODS**

**Plant Materials:**

The fruit pulp of *Tamarindus indica* Linn were collected from local Market in Khartoum - Sudan and authenticated by the botanist in the Medicinal and Aromatic Plants Research Institutes (MARPI), National Centre for Research, Khartoum – Sudan.

**Ethanolic extract of the fruit pulp of *T. indica*:**

Fruit pulp of *T. Indica* was shade dried and then made into powder form and was soaked in 500 ml of 80% ethanol for 24 hrs at room temperature, then the extract was filtered through filter paper and solvent was evaporated under reduced pressure using rotary evaporator apparatus (Trease and Evans 1983).
Animals:
Thirty Wister rats (130 – 150g) were obtained from the Medicinal and Aromatic Plant Research Institute, National Centre for Research, Khartoum, Sudan. The animals were kept, at the premises of the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum, under standard environmental conditions, controlled temperature (22± 2°C) and relative humidity (60%) with food and water provided ad libitum.

The research was carried out according to the rules governing the use of laboratory animals as acceptable international.

Acute Toxicity Test:
The acute toxicity test for T. indica ethanolic extract was performed using Wistar rats. The extract was administrated orally in increasing dose 150, 300 and 600mg/kg and rats were observed for 24 hrs.

Hepatoprotective Protocol:
Rats were divided into 5 groups of 6 rats each. Rats in group1 were given vehicle saline orally at 1ml/kg/day for 5 days and served as normal control, rats in group 2, were injected s/cu with CCl₄ at 0.2ml/kg diluted (1:9) in liquid paraffin on day 2 and 3 and received saline orally for 5 days. Rats in group 3 were administered orally with Silymarin at 50 mg/kg/day for 5 days and on days 2 and 3 injected with CCl₄ at 0.2 ml/kg diluted (1:9) in liquid paraffin. Rats in group 4 were administered orally with 150 mg/kg ethanolic extract of T. indica respectively, for 5 days and on days 2 and 3 injected with CCl₄ at 0.2 ml/kg diluted (1:9) in liquid paraffin. Group 5 was administrated orally with T. indica ethanolic extract alone at 150 mg/kg/day for 5 days.

Biochemical Analysis:
Serum was analyzed for the activities of aspartate aminotransferase (AST) Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and for the concentration of total bilirubin, total protein and albumin using commercial Kits (Biosystems, S. A. Costa Brava 30, Barcelona Spain).

Haematological Methods:
Haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cells count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured by the method of Schalm et al., (1975).

Histopathological Methods:
The liver specimens were collected and fixed in 10% formal saline, embedded in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin (H&E) for histopathological examination.

Statistical Methods:
The data are expressed as mean ± S.E. M. The difference among means has been analyzed by one way ANOVA, student's test was used for determining significance (Woolson, 1987). Difference considered significantly at (P<0.05).

Results:
Acute Toxicity Test:
At the doses tested (150, 300 and 600 mg/kg) on 24 hrs observations, the ethanolic extract of T.indica showed no mortalities and no abnormal clinical signs up to 600mg/kg.

Effects Of The Extract On Biochemical Parameters:
The values of AST, ALT, ALP, total bilirubin, total protein and albumin in serum of rats treated with ethanolic extract of T. indica were shown in Table 1. In carbon tetrachloride intoxicated rats the values of AST, ALT, ALP and bilirubin were significantly reduced when compared to the normal control. In the group intoxicated with CCI₄ and treated with 150 mg/kg of T. indica as well as in the Silymarin group, the values of the enzymes AST, ALT, ALP and the concentration of bilirubin were significantly reduced when compared to CCI₄ group and the values of total protein and albumin were slightly increased while there were no significant difference between the values of the serum enzymes in addition to bilirubin, total protein and albumin in the group treated with the extract alone (150 mg/kg) when compared to the normal control.
Effects Of The Extract On The Haematological Values:
Hb, PCV, RBC and MCHC were significantly decreased and the values of MCV were significantly increased in CCl4 treated group when compared to normal control while the values of these parameters were significantly increased in the groups treated with extract together with CCl4 and the group treated with Silymarin, except the values of MCV which was decreased when compared to the CCl4 group while there was no change in these haematological parameters in the group treated with the extract alone when compared to the normal control (Table 2).

### Table 1: Effects of *Tamarindus indica* ethanolic extract on serum biochemical parameters of Wistar rats intoxicated with CCl4

<table>
<thead>
<tr>
<th>Treatments</th>
<th>AST (I. u)</th>
<th>ALT (I. u)</th>
<th>ALP (I. u)</th>
<th>Total bilirubin (mg/dL)</th>
<th>Total Protein (g/dL)</th>
<th>Albumin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (control)</td>
<td>177.7±5.23</td>
<td>13.33±1.35</td>
<td>131.71±4.53</td>
<td>0.08±0.01</td>
<td>6.88±1.21</td>
<td>2.47±1.01</td>
</tr>
<tr>
<td>G2 (CCl4)</td>
<td>327.33±7.4*</td>
<td>27.72±2.03</td>
<td>259.82±6.11</td>
<td>0.25±0.11</td>
<td>5.86±1.02*</td>
<td>2.11±0.81*</td>
</tr>
<tr>
<td>G3 (CCl4 + Silymarin)</td>
<td>219.75±5.63</td>
<td>12.57±1.15</td>
<td>216.53±4.32</td>
<td>0.11±0.03</td>
<td>6.98±2.03*</td>
<td>2.96±0.72*</td>
</tr>
<tr>
<td>G4 (CCl4 + 150 mg/kg <em>T. indica</em>)</td>
<td>238.92±7.19</td>
<td>17.42±2.54</td>
<td>225.23±5.12</td>
<td>0.13±0.02</td>
<td>7.05±1.71</td>
<td>2.71±1.31</td>
</tr>
<tr>
<td>G5 (150 mg/kg <em>T. indica</em>)</td>
<td>197.77±6.33</td>
<td>15.61±2.71</td>
<td>148.87±4.71</td>
<td>0.07±0.01*</td>
<td>6.75±1.20*</td>
<td>2.57±1.15*</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M
**Significant level: P<0.05 compared to normal group (control)**
*Significant level: P<0.05 compared to CCl4 group
NS: not significant compared to normal group (control)

### Table 2: Effects of *Tamarindus indica* ethanolic extract on Hematological parameters of Wistar rats intoxicated with CCl4

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Hb (g/dL)</th>
<th>PCV (%)</th>
<th>RBC (× 10⁶)</th>
<th>MCV (fl)</th>
<th>MCHC(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 (control)</td>
<td>16.55±1.21</td>
<td>39.33±2.13</td>
<td>7.57±0.72</td>
<td>51.95±3.11</td>
<td>41.95±2.25</td>
</tr>
<tr>
<td>G2 (CCl4)</td>
<td>14.00±0.53</td>
<td>33.51±2.51</td>
<td>6.00±0.55</td>
<td>55.83±2.53</td>
<td>32.79±2.73</td>
</tr>
<tr>
<td>G3 (CCl4 + Silymarin)</td>
<td>15.52±0.75</td>
<td>38.25±4.15</td>
<td>7.23±1.02</td>
<td>53.12±4.44</td>
<td>37.90±3.52</td>
</tr>
<tr>
<td>G4 (CCl4 + 150 mg/kg <em>T. indica</em>)</td>
<td>15.33±1.05</td>
<td>41.66±4.93</td>
<td>6.65±0.63</td>
<td>53.65±5.17</td>
<td>38.39±1.94</td>
</tr>
<tr>
<td>G5 (150 mg/kg <em>T. indica</em>)</td>
<td>16.53±1.34</td>
<td>38.34±3.57</td>
<td>7.25±0.4</td>
<td>52.85±3.36</td>
<td>42.03±2.26</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M
**Significant level: P<0.05 compared to normal group
*Significant level: P<0.05 compared to CCl4 group
NS: not significant compared to normal group

### Histopathological Observations:

Fig. 1(A-D) showing histopathological liver sections of rats treated with CCl4 and/or Silymarin and *T. indica*. Severe vacuolization and necrotic hepatocytes were clearly appeared in CCl4 intoxicated group (Fig 1-A). (Fig 1-B) showed the liver sections of rats treated with CCl4 and Silymarin which manifested slight fatty changes. (Fig 1-C) liver cells of rats treated with CCl4 and 150 mg/kg *T. indica* also showed slight fatty changes. In group 5 which treated with the extract alone, liver sections showed normal architecture of the hepatocytes, (Fig 1-D).

### Discussion:

The results of the present study showed that the ethanolic extract of *T. indica* fruits possess hepatoprotective ingredients. CCl4 induced hepatotoxicity is the common experimental model for hepatoprotective drug screening. CCl4 is metabolizing to the trimethyl radical (CCl3) and a proxy trichlomethyl radical (OCOCll) by cytochrome P 450 2EI enzyme (Jia et al., 2011), these radicals bind covalently to the macromolecules and cause peroxidative degradation of cellular lipid membrane, which will cause the loss of integrity of all membranes and necrosis of hepatocytes (Ranawat et al., 2010).

In this study administration of *T. indica* at 150 mg/kg to CCl4 intoxicated rats reduced CCl4 induced elevation of serum AST, ALT, ALP and bilirubin and increased total protein. The lowering of enzyme level is definite indicator of hepatoprotective action and this protection is somewhat near to the protection produced by Silymarin although Silymarin is slightly better. Histopathological sections in extract treated group revealed less damage to the hepatic cells as compared to rats treated with CCl4.
Fig. 1: Liver cells of rats treated with ethanolic extract of Tamarindus indica alone and/or CCL₄ (A) Liver cells of rats treated with CCL₄ showed necrosis and massive fatty changes. (B) Liver cells of rats treated with CCL₄ and Silymarin showed slight fatty changes. (C) Liver cells of rats treated with CCL₄ and 150 mg/kg T. Indica showed slight fatty changes. (D) Liver cells of rats treated with 150 mg/kg T. Indica showed normal liver architecture.

The ability of hepatoprotective drugs to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms that have been disturbed by a hepatotoxin is the index of its protective effects (Yadav and Dixit 2003). The results of histopathological study support the results of biochemical parameters.

Normalization of CCl₄ damaged liver architecture by medicinal plants was indicated by many researchers; Nwigwe et al., 2012, Adinarayana et al., 2011 and Somchit et al., 2005 reported normalization of the damaged hepatocytes by Olax viridis, Tephrosia calophylla and Curcuma longa respectively.

There is no detailed published report on the hepatoprotective activity of T.indica so an initial dose of 150 mg/kg of the ethanolic extract was used. Herbal preparations were effective for the treatment of liver disorders (Karan et al., 1999; Chaterjee, 2000).

In this study the improvement of liver functions which was evident by decreased level of enzyme AST, ALT and ALP and bilirubin together with histopathology was in line with the results obtained by Elhag et al., (2011) and Ali et al., (2011) when treated CCl₄ intoxicated rats with Solanum nigrum and Khaya senegalensis respectively.

Amelioration effects of medicinal plants against hepatic damage induced by CCl₄ was proved by many authors; Helen Kadiri et al., 2007, reported protective effect of Nauclea pobeguinii, Friday et al., 2012, reported hepatoprotective effect of Ocimum gratissimum; Dhanasekaran and Ganapathy, 2011 reported the same effect of
Cassia auriculata; Samadram et al., 2008 indicated protective activity of leaves of Melia azadarach and seeds of Piper longum.

Daniyan and Muhammad, (2008), reported the presence of moderated concentration of tannins and alkaloids and low concentration of flavonoids and saponin in the ethanolic extract of the fruit pulp of T. indica, the presence of these compounds could be responsible for the membrane stabilizing activity.

The result of this study indicated that there was no significant alteration in both serum albumin and total protein in the group treated with the plant alone and this result was in line with the result obtained by Abukakar et al. (2008)

The reduction in RBC, Hb and PCV in CCl4 intoxicated group was improved to in the rats treated with the ethanolic extract and this was similar to the results obtained by Meral and Kanter (2003) who reported, increase levels of RBC, Hb and PCV in rats treated with Nigella sativa against CCl4 toxicity. This improvement in haematological parameters in this study may be due to active constituents in the fruit pulp of T. indica.

Conclusion:

We conclude that the ethanolic extract of Tamarindus indica fruit pulp possess hepatoprotective ingredients.

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