Hepatoprotective Activity of Methanol Extract of *Tecomella undulata* against Alcohol and Paracetamol Induced Hepatotoxicity in Rats

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**Abstract**

Plant products play a crucial role in the hepatoprotection through its antioxidants property. Therefore, search for modern medicine of plant origin with this property has become a central focus on hepatoprotection today. This study investigated to search a new hepatoprotective agent from natural sources, the methanol extract of folk medicinal plant, *T. undulata* leaves was tested against liver damage of albino rats. Levels of serum marker enzymes i.e. AST, ALT (aminotransferases), ALP (alkaline phosphatase), GGT (gamma glutamyl transpeptidase) and total bilirubin in serum alongwith the activities of LPO (lipid peroxidation), SOD (superoxide dismutase), CAT (catalase), GSH (reduced glutathione) and GPx (glutathione peroxidase) in liver homogenate from treated rats were monitored, respectively. The histopathological changes of liver sections were also compared with the respective controls. 30% alcohol and paracetamol induced significant (*P*<0.001) increase in thiobarbituric acid reactive substances (TBARS) alongwith the alterations in the activities of enzymatic and non-enzymatic antioxidants and serum markers in the liver and serum of treated rats. Simultaneously, oral treatment with *T. undulata* reversed to all the serum and liver parameters, dose-dependently, in 30% alcohol and paracetamol treated rats. The biochemical results were also compared with the standard drug- silymarin. These findings indicate the hepatoprotective potential of *T. undulata* leaves against liver damage might be due to the presence of flavonoids, quinones and other bioactive constituents.

**Keywords:** Liver damage; antioxidants; *Tecomella undulate*; hepatoprotection.

1. Introduction

Hepatic injury occurs due to infections, certain drugs, environmental and social factors such as alcoholism, and so forth [1]. Hepatic cells participate in the detoxification processes of the body and thus become vulnerable for damage through free radical generation [2]. Reactive oxygen free radicals have been known to produce tissue injury through covalent binding and lipid peroxidation and have been shown to augment fibrosis as seen from increased collagen synthesis [3]. Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues [4]. In recent years, the evaluation of hepatoprotective activity through antioxidant action is a central focus in the herbal drugs research. The currently observed rapid increase in consumption of herbal remedies worldwide has been stimulated by several factors, including the notion that all herbal products are safe and effective [5].

*Tecomella undulata* (Bignoniaceae), is a locally known as Rohida, found in Thar Desert regions of northwest and Western India. *T. undulata* plays an important role in environmental conservation and the leaves and stem bark are traditionally used as a remedy for syphilis, urinary disorders, enlargement of spleen, gonorrhoea, leucoderma and liver diseases [6]. Seeds are used against abscess [7]. The medicinal properties of *Tecomella undulata* (Rohida), particularly for the cure of jaundice, has been published by the local newspaper i.e. Dainik Bhaskar, a daily Hindi newspaper of North India, dated 3rd March 2006. Experimentally, the whole plant showed analgesic and anti-inflammatory activity [8] and the stem bark with other herbal formulations offered a hepatoprotective activity [9, 10].
Silymarin, a mixture of three flavonolignans - silybin, silidianin, and silychristine - from the seeds of 'milk thistle' (Silybum marianum) and has an excellent hepatoprotective action. Silymarin has been used medicinally to treat liver disorders, including cirrhosis and alcoholic liver diseases and cancers. Silymarin also has anti-inflammatory/anti-arthritis activities, antioxidant activities, immunomodulatory effects, etc. [11].

2. Methods

2.1. Plant Material and Extraction

The leaves of Tecoma undulata (T. undulate) used in this study were collected from Goluvala village, Shri Ganga Nagar District, Rajasthan, India, during the month of November 2007 and authenticated by Prof. N.J. Sarna, Department of Botany, University of Rajasthan, Jaipur, where a voucher specimen has been preserved for future identification. The leaves of plant were shade-dried and pulverized. The powder was treated with petroleum ether for defatting as well as to remove chlorophyll. The powder was packed into a Soxhlet apparatus and subjected to hot continuous percolation using methanol (95% v/v) as solvent. The extract was concentrated under vacuum and dried in a vacuum desiccator (yield 7.8% w/w). After that it was used for oral experimentation, to evaluate the antioxidant and hepatoprotective activity.

2.2. Animals

Wistar albino male rats (150-200 g each), maintained under standard animal housing conditions (12 h light and dark cycle), were used for all sets of experiment. The rats were allowed standard laboratory feed and water ad libitum.

2.3. Acute Toxicity

The leaf extract was administered to the test groups in graded doses ranging up to 4 g / kg body wt. and the rats were observed for signs of toxicity and mortality for 15 days afterward. The minimum dose levels i.e. 100 and 200 mg / kg were used for experimentation [12].

2.4. Treatment Design - I

2.4.1. Alcohol-Induced Experimental Liver Damage

All rats were divided into 5 groups of 6 rats each:

**Group I:** Vehicle treated rats were kept on normal diet and served as control for 15 days.
**Group II:** Rats orally received 30% alcohol (1.5 ml/rat / twice a day) for 15 days.
**Group III:** Rats orally received Silymarin (25 mg/kg b. wt/day) and alcohol as group II, for 15 days.
**Group IV:** Rats orally received T. undulata extract (100 mg/kg b. wt / day) and alcohol as group II, for 15 days.
**Group V:** Rats orally received T. undulata extract (200 mg/kg b. wt /day) and alcohol as group II, for 15 days.

2.5. Treatment Design - II

2.5.1. Paracetamol-Induced Experimental Liver Damage

In case of paracetamol-induced hepatotoxicity, the rats were divided into 5 groups of 6 rats each:

**Group I:** Vehicle treated rats were kept on normal diet and served as control for 15 days.
**Group II:** Rats received paracetamol (500 mg/kg b. wt /day, orally) for 15 days.
**Group III:** Rats received silymarin (25 mg/kg b. wt /day, orally) and paracetamol as group II, for 15 days.
**Group IV:** Rats received T. undulata extract (100 mg/kg b. wt /day) and paracetamol as group II, for 15 days.
**Group V:** Rats received T. undulata extract (200 mg/kg b. wt /day, orally) and paracetamol as group II, for 15 days.
2.6. Assessment of Liver Functions

After the last dose-delivery, all rats were kept on starved condition for 24 hours, after that autopsied and blood was collected by cardiac puncture of all the experimental rats. Serum was separated and analyzed for various biochemical parameters like AST, ALT [13], ALP [14], GGT [15] and total bilirubin [16].

The livers were examined grossly, weighed, and then half of the liver was used for biochemical estimations like LPO [17], SOD [18], CAT [19], GSH [20] and GPx [21], respectively. Remaining half of the liver was fixed in Bouin’s fluids after that processed for paraffin embedding using the standard micro technique [22]. Sections of the liver (5 µm) were prepared and stained with haematoxylin and eosin for histopathological studies.

2.7. Statistical Analysis

All values were expressed as mean ± SEM. Data were analyzed by applying student’s t-test.

3. Results

Administration of 30% alcohol and paracetamol to rats for the period of 15 days caused significant (p<0.001) rise in the levels of AST, ALT, ALP, GGT and total bilirubin in serum when compared with normal controls (Table 1 and 2). The effect of T. undulata leaves extract on serum-AST, ALT, ALP, GGT and total bilirubin in 30% alcohol and paracetamol treated rats showed significant dose-dependent (p<0.001; p<0.01; p<0.05, respectively) decline as compared to 30% alcohol and paracetamol treated groups. The degree of protection by T. undulata leaves (200 mg/kg) was observed statistically similar with the standard drug (Table 1 and 2).

Table 1 and 2 showing a significant (p<0.001) increase in the level of hepatic LPO in 30% alcohol and paracetamol treated rats as compared to normal controls. In contrast, treatment with T. undulata leaves extract significantly (p<0.05; p<0.01; p<0.001) prevented this rise in LPO level at 100 and 200 mg/kg dose levels. The protection of LPO by T. undulata leaves extract (200 mg/kg) and silymarin was found to be statistically equal against 30% alcohol and paracetamol-induced lipid peroxidation (Table 1 and 2).

Table 1 and 2 depict that rats treated with 30% alcohol and paracetamol caused a significant (p<0.001) decline in the hepatic antioxidants such as SOD, CAT, GSH and GPx in comparison to normal controls. Simultaneously, oral treatment with T. undulata at the dose levels 100 and 200 mg/kg body weight/day showed significant (p<0.05; p<0.01; p<0.001) elevation in the activity of all antioxidant parameters like SOD, CAT, GSH and GPx. The elimination of hepatic oxidative stress by T. undulata leaves extract (200 mg/kg) and silymarin (25 mg/kg) was statistically almost similar in nature (Table 1 and 2).

Histopathological examination of the liver section of rats treated with toxicant showed intense centrilobular necrosis and vacuolization. The rats treated with the extract and silymarin alongwith toxicant showed signs of protection against these toxicants to a considerable extent as evident from formation of normal hepatic cords and absence of necrosis and vacuoles.

4. Discussion

Hepatic cells appear to participate in a variety of enzymatic metabolic activities and both alcohol and paracetamol produced marked liver damage at the given doses as expected [23]. Formation of reactive oxygen species (ROS) oxidative stress and hepatocellular injury have been implicated to alcoholic liver disease. It has been documented that Kupffer cells are the major sources of ROS during chronic alcohol consumption, and these are primed and activated for enhanced formation of pro-inflammatory factors [24]. Additionally, alcohol-induced liver injury has
been associated with increased amount of lipid peroxidation [25]. It may thus be plausible that in our study, loss of membrane structure and integrity because of lipid peroxidation was accompanied with the elevated levels of marker enzymes like-AST, ALT, ALP, GGT and total bilirubin. Indeed, \textit{T. undulata} supplementation in our study, was potentially effective in blunting lipid peroxidation, suggesting that the extract possibly has antioxidant property to reduce ethanol-induced membrane lipid peroxidation and thereby to preserve membrane structure might be due to the presence of glycosides, flavonoids, proteins, amino acids, tannins, saponins and triterpenoids [26].

Our study further revealed that chronic exposure to alcohol, decreased the activities of the ROS scavenging enzymes, viz., SOD and CAT. This is in line with assumption suggested earlier by Balasubramaniyan et al. [27] that decrease in the activity of antioxidants SOD, CAT, GSH and GPx following alcohol exposure may be due to the damaging effects of free radicals or alternatively could be due to a direct effect of acetaldehyde, formed from oxidation of alcohol [28]. In our studies, \textit{T. undulata} supplementation could restore the activity of these antioxidants and possibly could reduce the generation of free radicals and hepatocellular damage by the presence of some quinones, stigmasterol and β-sitosterol which are experimentally well known for their antioxidant activity [29, 30].

Paracetamol in larger doses produces liver necrosis after undergoing bio-activation to a toxic electrophile, N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome \textit{P}-450 mono-oxygenase [31]. NAPQI binds to macromolecules and cellular proteins, and also oxidizes lipids and alters homeostasis of calcium after depletion of SOD, CAT, GSH and GPx. The supplementation of \textit{T. undulata} extract restored the depleted SOD, CAT, GSH and GPx contents near normalcy and also brought down to elevated levels of AST, ALT, ALP, GGT and total bilirubin. These biochemical restorations may be due to the inhibitory effects on cytochrome \textit{P}-450 or /and promotion of its glucuronidation [32]. Histopathological analysis of the liver sections is in good agreement with biochemical changes. Therefore, on the basis of our results, the possible mechanism of hepatoprotective effect through antioxidant activity of \textit{T. undulata} might be due to the presence of flavonoids, quinones and other active constituents. Further studies are in progress to isolate the active constituents of \textit{T. undulata} and also to evaluate the exact mechanism of action for the hepatoprotective activity through its antioxidant potential.

\textbf{Competing Interests}

Authors declare that they have no competing interests.

\textbf{Authors’ Contributions}

All authors contributed equally to this work.

\textbf{Acknowledgement}

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\textbf{References}


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Table 1: Showing antioxidant and hepatoprotective activity of T. undulata leaves extract on alcohol-induced liver damage in rats

<table>
<thead>
<tr>
<th>Treatment design</th>
<th>Serum AST (IU/L)</th>
<th>Serum ALT (IU/L)</th>
<th>Serum ALP (KAU)</th>
<th>Serum total bilirubin (mg/100 ml)</th>
<th>Liver GSH (μ mole/g tissue)</th>
<th>Liver SOD (μ mole/mg protein)</th>
<th>Liver CAT (μ mole H2O2 consumed/min/mg protein)</th>
<th>Liver GPx (μ mole NADPH consumed/min/mg protein)</th>
<th>Liver LPO (μ mole MDA/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group I)</td>
<td>128.32±3.76</td>
<td>105.46±3.34</td>
<td>21.16±1.39</td>
<td>0.69±0.07</td>
<td>4.45±0.30</td>
<td>9.54±0.92</td>
<td>63.15±3.98</td>
<td>12.88±0.41</td>
<td>1.72±0.15</td>
</tr>
<tr>
<td>Alcohol (1.5 ml/Rat/day) (Group II)</td>
<td>523.44±5.41*</td>
<td>431.13±5.36*</td>
<td>46.21±3.21*</td>
<td>32.10±2.12*</td>
<td>2.33±0.29</td>
<td>1.45±0.21*</td>
<td>27.15±1.52*</td>
<td>5.88±0.22*</td>
<td>5.16±0.72*</td>
</tr>
<tr>
<td>Silymarin + Alcohol (50 mg/kg b wt) (Group III)</td>
<td>152.21±3.48*</td>
<td>124.10±3.32*</td>
<td>28.15±1.29*</td>
<td>14.42±0.32*</td>
<td>0.98±0.09*</td>
<td>4.10±0.32*</td>
<td>60.12±4.03*</td>
<td>10.42±0.29*</td>
<td>2.19±0.23*</td>
</tr>
<tr>
<td>T. undulata + Alcohol (100 mg/kg b wt) (Group IV)</td>
<td>310.12±4.15*</td>
<td>245.17±4.32*</td>
<td>37.21±2.24*</td>
<td>21.10±1.08*</td>
<td>1.42±0.12*</td>
<td>2.49±0.19*</td>
<td>47.10±2.17*</td>
<td>7.78±0.19*</td>
<td>4.10±0.14*</td>
</tr>
<tr>
<td>T. Undulata + Alcohol (200 mg/kg b wt) (Group V)</td>
<td>163.14±4.27*</td>
<td>127.13±3.78*</td>
<td>24.16±1.72*</td>
<td>12.12±0.42*</td>
<td>0.77±0.16*</td>
<td>4.15±0.29*</td>
<td>65.13±2.10*</td>
<td>9.92±0.31*</td>
<td>2.39±0.12*</td>
</tr>
</tbody>
</table>

Significance level:  

\[ a = P \leq 0.001 \]  
\[ b = P \leq 0.01 \]  
\[ c = P \leq 0.05 \]  

Data are mean ± SEM (n = 6). Group II compared with control (Group I). Group III, IV and V compared with Group II.
Table 2: Showing antioxidant and hepatoprotective activity of *T. undulata* leaves extract on paracetamol-induced liver damage in rats

<table>
<thead>
<tr>
<th>Treatment design</th>
<th>Serum AST (IU/L)</th>
<th>Serum ALT (IU/L)</th>
<th>Serum ALP (KAU)</th>
<th>Serum total bilirubin (mg/100 ml)</th>
<th>Liver GSH (μ mole/g tissue)</th>
<th>Liver SOD (μ mole/mg protein)</th>
<th>Liver CAT (μ mole H₂O₂ consumed/min/mg protein)</th>
<th>Liver GPs (n mole NADPH consumed/min/mg protein)</th>
<th>Liver LPO (n mole MDA/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Group I)</td>
<td>112.18±3.09</td>
<td>93.13±2.89</td>
<td>18.21±1.10</td>
<td>8.33±0.27</td>
<td>4.38±0.21</td>
<td>8.42±0.76</td>
<td>60.12±3.49</td>
<td>13.42±0.28</td>
<td>1.85±0.14</td>
</tr>
<tr>
<td>Paracetamol (500 mg/kg b wt) (Group II)</td>
<td>415.20±5.56*a</td>
<td>292.10±4.21*a</td>
<td>35.18±2.15*a</td>
<td>22.12±1.19*a</td>
<td>2.46±0.39*a</td>
<td>1.27±0.18*a</td>
<td>4.50±0.52*a</td>
<td>32.16±2.17*a</td>
<td>6.21±0.12*a</td>
</tr>
<tr>
<td>Silymarin + Paracetamol (50 mg/kg b wt) (Group III)</td>
<td>138.10±4.12*a</td>
<td>117.14±3.15*a</td>
<td>23.10±1.42*a</td>
<td>10.21±0.79*a</td>
<td>1.06±0.11*a</td>
<td>3.78±0.29*a</td>
<td>7.30±0.33*a</td>
<td>54.18±3.63*a</td>
<td>11.21±0.34*a</td>
</tr>
<tr>
<td><em>T. undulata</em> + Paracetamol (100 mg/kg b wt) (Group IV)</td>
<td>261.78±4.59*a</td>
<td>205±3.89*a</td>
<td>27.13±1.49*a</td>
<td>18.33±1.10*a</td>
<td>1.74±0.17*b</td>
<td>2.12±0.24*b</td>
<td>6.08±0.42*b</td>
<td>43.16±2.69*b</td>
<td>8.22±0.19*b</td>
</tr>
<tr>
<td><em>T. Undulata</em> + Paracetamol (200 mg/kg b wt) (Group V)</td>
<td>142.13±3.79*a</td>
<td>116.12±4.27*a</td>
<td>22.72±1.33*a</td>
<td>11.31±0.41*a</td>
<td>0.93±0.13*a</td>
<td>4.15±0.27*a</td>
<td>7.79±0.29*a</td>
<td>58.17±3.12*a</td>
<td>10.29±0.16*a</td>
</tr>
</tbody>
</table>

Significance level:  

- a = P ≤ 0.001  
- b = P ≤ 0.01  
- c = P ≤ 0.05  

Date are mean ± SEM (n = 6)


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