Assessment of hepatoprotective effect of *Tecomella undulata* on paracetamol-induced hepatotoxicity in rats

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Abstract: The aim of the present study was to validate the hepatoprotective activity of bark of *Tecomella undulata* (Sm.) Seem., Biognoniaceae, against paracetamol (PCM) induced hepatic damage. Chloroform soluble fraction (Fraction-I), acetone soluble fraction (Fraction-II), methanol soluble fraction (Fraction-III) and methanol insoluble fraction (Fraction-IV) of ethanolic extract of bark of *T. undulata* were evaluated for hepatoprotective activity against paracetamol induced hepatic damage using biochemical, morphological, functional and histopathological studies. The methanol soluble fraction (Fraction-III) was most potent among the four fractions studied in detail. Fraction-III showed significant hepatoprotective activity against paracetamol induced hepatic damage as evident by normalization of substantially elevated levels of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and total bilirubin (TBil), decreased level of total protein (TP), increased wet liver weight and volume, increased thiopentone sodium induced sleeping time and abnormal histopathology. Present study showed that the Fraction-III of ethanolic extract of bark of *T. undulata* significantly restores physiological integrity of hepatocytes. Fraction-III did not show any sign of toxicity up to oral dose of 1500 mg/kg in mice.

Introduction

Management of liver diseases is still a challenge to the modern medicine. Modern medicines have little to offer for alleviation of hepatic ailments, whereas most important representatives are of phytoconstituents (Sallie et al., 1991). About 170 phytoconstituents isolated from 110 plants belonging to 55 families are stated to possess liver protective activity (Handa et al., 1986). *Tecomella undulata* (Sm.) Seem., Bignoniaceae, is a deciduous or nearly evergreen large ornamental tree of arid and semi arid regions (Chopra et al., 1992) and known as Rohida in Hindi (The Ayurvedic Pharmacopoeia of India, 2004). Plant has anti-bacterial (Gehlot et al., 2000; Parekh et al., 2007), analgesic (Ahmad et al., 1994), anti-AIDS (Azam, 1999), anti-oxidant (Gautam et al., 2007) and hepatoprotective activity (Khatri et al., 2009; Rana et al., 2008).

In the ethnobotanical claims, the bark is used for the treatment of jaundice and other hepatic diseases by the folk tribes of Rajasthan state, India (Punjani & Kumar, 2003). There is no scientific report available in support of the hepatoprotective activity of *Tecomella undulata* bark on paracetamol-induced hepatotoxicity in rats.

Materials and Methods

Drugs and chemicals

Paracetamol was procured from Rajasthan Drug Pharmaceuticals Limited (RDPL), Jaipur. Silybon-140 tablets (Micro Labs) of silymarin and Thiosol vial (Neon Labs) of thiopentone sodium were purchased from the local market. Standard kits (Logotech India Pvt. Ltd, New Delhi, India) were used for determination of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total bilirubin (TBil) and total protein (TP).

Plant material

The bark of *Tecomella undulata* (Sm.) Seem.,
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Biognoniaceae, was procured from Cazri region of Jodhpur, Rajasthan, India in the month of October. The identity of the plant was confirmed at The Botanical Survey of India, Government of India, Ministry of environment & forest, Jodhpur. The voucher specimen (No. BSI/AZC/1.12012/Tech-2005/17) was deposited at the herbarium of Botanical Survey of India, Jodhpur.

Experimental animals

Albino mice 20-40 g and Wistar rats 140-160 g of either sex were used to carry out acute toxicity studies and hepatoprotective activity respectively. They were obtained from the animal house, Indian Institute of Veterinary Science, Izatnagar (U.P), India. The approval of experimental protocols of animal studies was taken from Institution Animal Ethics Committee (LMC/PHARM/OFFICE/2008-09/835/CPCSEA). The animals were grouped and housed in polycrylic cages (38 x 23 x 10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 27±2 °C) with light and dark cycle (12/12 h). The animals were fed with standard pellet diet (Lipton India Ltd., Mumbai, India) and fresh water ad libitum. All the animals were acclimatized to laboratory condition for a week before commencement of experiment.

Preparation of extract

The bark was cut into small pieces; shade dried; powdered with a mechanical grinder to obtain a coarse powder. About 125 g powdered drug was subject to defating with petroleum ether (1200 mL x 2). The defatted material (marc) was extracted with 95% ethanol (1200 mL x 4) and resulting ethanolic extract was concentrated and vacuum dried. The dried total ethanolic extract was fractionated successively with chloroform (500 mL x 2), acetone (500 mL x 2) and methanol (500 mL x 2). All the fractions; chloroform soluble fraction, acetone soluble fraction, methanol soluble fraction and methanol insoluble fraction, were concentrated and dried under vacuum and were marked as Fraction-I, Fraction-II, Fraction-III and Fraction-IV respectively and were used for evaluation of hepatoprotective activities.

Preliminary phytochemical investigation

The different fractions of ethanolic extract of Tecomella undulata bark were subjected for phytochemical study (Harbone, 1979).

Acute toxicity studies

The acute toxicity studies for different fractions of ethanolic extract of Tecomella undulata bark were performed as per stair case method using albino mice (Ghosh, 1984). The animals were fasted overnight prior to the experiment and maintained under standard conditions. All the fractions were administrated orally in increasing dose and found safe up to dose of 2000, 2000, 1500 and 1000 mg/kg for Fraction-I, Fraction-II, Fraction-III and Fraction-IV respectively.

Paracetamol induced hepatotoxicity

Rats were divided into seven groups (n=6). Group I (Normal control) animals received once daily 2 mL/kg orally 2% gum acacia suspension for four days and single dose of sucrose solution 2 mL/kg orally on day three. Group II (PCM control) animals received once daily 2 mL/kg orally 2% gum acacia suspension for four days and single dose of 2 mL/kg orally PCM (2 g/kg) suspension in sucrose on day three. Group III (standard) animals received once daily 2 mL/kg orally standard drug Silymarin (100 mg/kg) suspension in 2% gum acacia for four days. Test groups animals (Groups IV–VII) received once daily 2 mL/kg orally Fraction-I (200 mg/kg), Fraction-II (200 mg/kg), Fraction-III (150 mg/kg) and Fraction-IV (100 mg/kg) suspension in 2% gum acacia for four days respectively. The groups III-VII animals were administered simultaneously single dose of 2 mL/kg orally PCM (2 g/kg) suspension in 2% gum acacia on day three after 30 min. of administration of the Silymarin, Fraction-I, Fraction-II, Fraction-III and Fraction-IV respectively. On the day 5th, aqueous solution of thiopentone sodium (37 mg/kg, i.p.) in water for injection was injected to the animals and the sleeping time was noted. After complete recovery from thiopentone sodium effect, blood was withdrawn by puncturing the retro-orbital. Blood was collected, allowed to clot and serum was separated at 2500 rpm for 15 min and biochemical investigations were carried out. Animals were sacrificed with excess of light ether anesthesia. Liver was dissected out, rinsed with water and used for morphological and histopathological studies.

Biochemical studies

The biochemical parameters AST (Reitman et al., 1957), ALT (Reitman et al., 1957), ALP (King, 1965), TBil (Malloy et al., 1937), TP (Thapa et al., 2007) were assayed.

Morphological studies

The morphological parameters, wet liver weight and volume were determined. The wet liver
weight was determined by using an electronic balance. The wet liver volume was determined by dropping the liver in a measuring cylinder containing a fixed volume of distilled water and the volume displaced was recorded.

Functional studies

Thiopentone sodium was used to determine the functional capacity of liver. The time interval between the loss and the regaining of the righting reflex was measured as sleeping time. Onset and duration of action of sleeping time was noted.

Histopathological studies

The dissected liver was fixed in 10% formalin, dehydrated in gradual isopropyl alcohol (60-100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hematoxylin and eosin (H-E) dye for photomicroscopic observation (Luna, 1968).

Statistical analysis

The values were expressed as mean±S.E.M. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnett test. *p* values <0.05 were considered significant.

Results

Phytochemical study

All the fractions of ethanolic extract of *Tecomella undulata* bark showed the red to brown colouration, characteristic odour and semi-solid like consistency. The percentage yields (% w/w) of Fraction-I, Fraction-II, Fraction-III and Fraction-IV were 7.2, 6.4, 7.9 and 2.6 respectively. Phytochemical analysis of fractions showed the presence of glycosides, flavonoids, proteins, amino acids, tannins, saponins, triterpenoids and gums.

Acute toxicity studies

The effective doses of Fraction-I, Fraction-II, Fraction-III and Fraction-IV were 200, 200, 150 and 100 mg/kg respectively.

Effect of fractions on ALT, AST, ALP, TBil, TP level

The result of the effect of fractions on biochemical parameters in PCM treated rats is shown in Table I. In the PCM treated rats, the level of AST, ALT, ALP and TBil were increased to 301.0±4.16 IU/L, 248.2±3.09 IU/L, 260.3±3.77 IU/L and 2.66±0.02 mg/dL respectively and TP decreased to 3.56±0.19 mg/dL, whereas these values were 128.5±2.26 IU/L, 93.2±3.82 IU/L, 145.8±4.69 IU/L, 1.01±0.02 mg/dL and 7.02±0.28 mg/dL in normal control rats respectively. On administration of Silymarin, Fraction-I, Fraction-II, Fraction-III and Fraction-IV in group III, IV, V, VI and VII respectively, the level of these biochemical parameters were found retrieving towards normalcy. Fraction-III of ethanolic extract showed significant activity in comparison to the other fractions.

Effects of fractions on wet liver weight and volume

The result of the effect of fractions on morphological parameters in PCM treated rats is shown in Table I. In the PCM treated rats, wet liver weight and liver volume were increased to 4.74±0.13 /100 g and 6.28±0.36 mL/100 g respectively whereas these values were 3.91±0.13 g/100 g and 3.87±0.25 mL/100 g in normal control rats respectively. On administration of Silymarin, Fraction-I, Fraction-II, Fraction-III and Fraction-IV in group III, IV, V, VI and VII respectively, the level of these morphological parameters were found retrieving towards normalcy. Fraction-III of ethanolic extract showed significant activity in comparison to the other fractions.

Effects of fractions on thiopentone sodium induced sleeping time

The result of the effect of fractions on functional parameters in PCM treated rats is shown in Table I. In the PCM treated rats, onset of action of sleeping time was decreased to 94.8±2.18 sec. and duration of action was increased to 154.6±6.38 min., whereas these values were 164.6±6.44 sec. and 77.6±5.32 min. in normal control rats respectively. On administration of Silymarin, Fraction-I, Fraction-II, Fraction-III and Fraction-IV in group III, IV, V, VI and VII respectively, the sleeping time were found retrieving towards normalcy. Fraction-III of ethanolic extract showed significant activity in comparison to the other fractions.

Histopathological observations

Histology of the liver sections of normal control rats showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus and visible central veins (Figure 1A) where as massive fatty changes, necrosis, ballooning degeneration, broad infiltration of the lymphocytes and the loss of cellular boundaries (Figure 1B) in the liver sections of PCM.
treated rats. The histological architecture of liver sections of the rats treated with fractions showed more or less normal lobular pattern with a mild degree of fatty changes, necrosis and lymphocyte infiltration (Figures 1D, E, F, G) almost comparable to the control and silymarin treated rats (Figure 1C).

Figure 1. A. Liver sections of normal control rats. B. Liver sections of PCM treated rats. C, D, E, F and G Liver sections of rats treated with PCM+Silymarin, PCM+Fraction-I, PCM+Fraction-II, PCM+Fraction-III and PCM+Fraction-IV respectively.
Discussion and Conclusion

In the present study, fractions of ethanolic extracts of Tecomella undulata bark were evaluated for the hepatoprotective activity using hepatotoxicity induced by PCM in rats. The hepatoprotective effect of fractions of ethanolic extract of Tecomella undulata bark was compared with Silymarin, which is an active constituent of the fruit of the milk thistle (Silybum marianum, Asteraceae). Liver damage was assessed by biochemical (AST, ALT, ALP, TBil and TP level), morphological (wet liver weight and volume), functional (sleeping time) and by histopathological studies. PCM-induced hepatic injury method is commonly used for the study of hepatoprotective effects of medicinal plants extracts and drugs. The PCM is widely used for the study of hepatoprotective activity of fractions of ethanolic extracts of bark against the toxic effect of PCM, which was also supported by morphological, functional and histological studies. The preliminary phytochemical analysis of the fractions has shown the presence of flavonoids, which has been known for their antioxidant and hepatoprotective activities (Di Carlo et al., 1999). Thus, it can be concluded that possible mechanism of hepatoprotective activity of Tecomella undulata bark may be due to its free radical-scavenging and antioxidant activity. Histopathological changes in the liver sections also reveal the process of regeneration and reduction the necrosis.

Thus the present study proves the hepatoprotective action of Tecomella undulata (Sm.) Seem., Biognoniaceae, bark extract against experimentally induced liver damage in the rats and justify its use in traditional folk medicine and hepatoprotective preparations for liver affection.

Acknowledgement

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References


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**Table 1. Effect of fractions on biochemical, morphological and functional parameters in PCM treated rats.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Biochemical parameters</th>
<th>Morphological parameters</th>
<th>Functional parameters</th>
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<tr>
<td></td>
<td>AST (IU/L)</td>
<td>ALT (IU/L)</td>
<td>ALP (mg/dL)</td>
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<tr>
<td>Group I</td>
<td>128.5±2.26</td>
<td>93.2±3.82</td>
<td>145.8±4.69</td>
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<tr>
<td>Group II</td>
<td>301.0±4.16</td>
<td>248.2±3.09</td>
<td>260.3±3.77</td>
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<td>Group III</td>
<td>143.7±2.93</td>
<td>105.5±3.03</td>
<td>151.5±3.38</td>
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<tr>
<td>Group IV</td>
<td>249.3±3.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>199.7±4.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>215.5±4.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V</td>
<td>185.2±6.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>158.5±4.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>191.3±3.77&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Group VI</td>
<td>140.0±3.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>111.3±2.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148.2±5.24&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Group VII</td>
<td>154.8±4.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>123.3±2.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>161.5±4.36&lt;sup&gt;a&lt;/sup&gt;</td>
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Results are presented as mean±SEM, (n=6) One-Way ANOVA followed Dunnett test. <sup>a</sup>p<0.05 significantly different from PCM treated group. <sup>b</sup>p<0.05 significantly different from Silymarin treated group.
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