# HEPATOPROTECTION OF *TECOMELLA UNDULATA* AGAINST EXPERIMENTALLY INDUCED LIVER INJURY IN RATS

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**Summary**

*Tecomella undulata* (Bignoniaceae) is tree species, locally known as Rohida, found in Thar Desert regions of northwest and western India. The bark obtained from the stem is used as a remedy for syphilis, urinary disorders, enlargement of spleen, gonorrhoea, leucoderma and liver diseases. The aim of this work is to study the hepatoprotective effect of crude Methanolic extract from the bark parts of *Tecomella undulata*. The methanolic extract obtained from bark parts of *Tecomella undulata* was evaluated for hepatoprotective activity in rats by inducing liver damage by carbon tetrachloride. The methanolic extract at an oral dose of 200 mg/kg exhibited a significant ($P < 0.05$) protective effect by lowering serum levels of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, total bilirubin and total cholesterol and increasing the levels of total protein and albumin levels as compared to silymarin used as a positive control. These biochemical observations were supplemented by histopathological examination of liver sections. The activity may be a result of the presence of flavonoid compounds. Furthermore, the acute toxicity of the extracts showed no signs of toxicity up to a dose level of 2000 mg/kg. Thus it could be concluded that methanolic extract of *Tecomella undulata* possesses significant hepatoprotective properties.

Keywords: *Tecomella undulata*; Carbon tetrachloride ($CCL_4$); Hepatoprotection; Silymarin.

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## Introduction

*Tecomella undulata* is tree species, locally known as Rohida, found in Thar Desert regions of northwest and western India and has long been used as a remedy for syphilis, urinary disorders, enlargement of spleen, gonorrhea, leucoderma, jaundice and liver diseases (1,2). The literature survey reveals that little work has been carried out on this plant. The plant is useful as antifungal and anti-termite properties (3), typhoid fever (4), analgesic and anti-inflammatory (5), antimicrobial activity (6,7), drug has been done against chlorpromazine drug in albino rats (8), non-specific spasmolytic action (9), Family Planning and Sex DiseaseTreatment in Samahni Valley, Pakistan (10) and a drug that is currently used for checking the spread of AIDS (11). Phytochemically the plant had been investigated for Chromone glucoside (12), lapachol (13), quinones (16), iridoid glucoside (14,15,16), tecomin (17,18), rutin, quercetin, luteolin glucoside (19), tecoside (21) and undulatin (20) have been reported from this plant. The present study was undertaken to scientifically prove the folklore use of the plant against liver disorders.

## Materials and methods

### Plant material

The bark parts of the *Tecomella undulata* were collected from the rigion of Pavijetpur, Gujarat state, India and their identity was conformed by Prof. Vishal Muliya, Department of Botany, Christ College, Rajkot and a voucher specimen was deposited.

### Preparation of extracts

The shade dried bark parts of about 500 g were subjected for size reduction to coarse powder. The powder was defatted with petroleum ether (60–80 ◦C) and then extracted with 5 l of 95% methyl alcohol using soxhlet apparatus till exhaustion for about 32 h. The methanolic extract was concentrated under vacuum to get the residues. The percentage yield of methanolic extract was found to be 17.6 % (w/w). Silymarin was used as a positive control at an oral dose of 200 mg/kg (22). All the test suspensions are prepared in vehicle, i.e., CMC.

### Animals

Wistar albino rats of either sex, weighing 200–250 g maintained under standard husbandry conditions (temperature 23±2 ◦C, relative humidity 55±10% and 12-h light: 12-h dark cycle) were used for all experiments. Animals were allowed to take standard laboratory feed and tap water. The experiments were performed after the experimental protocols approved by the institutional animal ethics committee, Saurashtra University, Rajkot, Gujarat.

### Toxicity studies

Acute toxicity study was performed for methanolic extract according to the acute toxic classic method (23). Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose of 300 mg/kg and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated again

to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose, i.e., 2000 mg/kg. One-tenth of the maximum dose of the extract tested for acute toxicity was selected for evaluation of hepatoprotective activity, i.e., 200 mg/kg (24).

**Carbon tetrachloride-induced hepatotoxicity in rats**

Rats were divided into five groups of six each, control, hepatotoxin, positive control and two test groups. The control group received oral vehicle treatment at 0, 24 and 48 h. The animals in hepatotoxin-treated group received vehicle at 0 h and at 24 h vehicle followed by carbon tetrachloride diluted in liquid paraffin (1:1, i.p.) at a dose of 1.25 ml/kg, while at 48 h these animals received only vehicle. The test groups have received the first dose of extracts at 0 h, second dose of extracts at 24 h, which was followed by a dose of carbon tetrachloride and at 48 h the third dose of extracts (25,26). The positive control group has received the first dose of silymarin (200 mg/kg) (22) at 0 h, at 24 h the second dose of Silymarin followed by a dose of carbon tetrachloride and at 48 h the third dose of silymarin. After 72 h blood was collected from all the groups, and allowed to clot for the separation of serum. The serum was used for estimation of biochemical parameters. Glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) are estimated by Reitman and Frankel Method (27), alkaline phosphatase (ALKP) by PNPP method (28), total bilirubin (TBL) by Jendrassik and Grof method (29), total cholesterol (CHL) by CHOD-PAP Method (30), total protein (TPTN) by colour complexation with copper ions in an alkali solution (31) and albumin (ALB) was estimated by Bromo Cresol Green Method (32). All the determinations were carried out using standard kits (Span diagnostic, Surat) using UV spectrophotometer (Shimadzu, UV-1700 Pharmaspectra, Japan).

**Histopathological studies**

One animal from each of the treated groups showing maximum activity as indicated by improved biochemical parameters was used for this purpose. The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12 h, then embedded in paraffin using conventional methods (33) and cut into 5_m thick sections and stained using haematoxylin–eosin dye and finally mounted in di-phenyl xylene. Then the sections were observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

**Statistical analysis**

The mean values ± S.E.M. are calculated for each parameter. For determining the significant inter-group difference each parameter was analyzed separately and one-way analysis of variance (ANOVA) (34) was carried out and the individual comparisons of the group mean values were done using Dunnet's Procedure (35).

## Results

The Methanolic extract did not cause any mortality up to 2000 mg/kg and were considered as safe (OECD, 1996). The rats which have received methanolic extract at the dose of 2000 mg/mg exhibited ptosis.

Carbon tetrachloride ($CCl_4$) intoxication in normal rats elevated the levels of SGOT, SGPT, ALKP, TBL and CHL, whereas decrease in the levels of TPTN and ALB were observed significantly indicating acute hepatocellular damage and biliary obstruction. The rats treated with methanolic extract (200 mg/kg) and also silymarin, showed a significant decrease in all the elevated SGOT, SGPT, ALKP, TBL and CHL levels and significant increase in TPTN and ALB levels (Table 1).

Table.1 Effect of *Tecomella undulata* on CCl4-induced toxicity in rats

| Group | SGOT (IU/I) | SGPT (IU/I) | ALKP (IU/I) | TB (mg/dl) | TC (mg/dl) | TP (mg/dl) | ALB (g/dl) |
|---|---|---|---|---|---|---|---|
| **Control** | 289.33 ± 9.23 | 51.5 ± 2.95 | 16.928 ± 0.36 | 1.017 ± 0.042 | 50.416 ± 0.649 | 6.534 ± 0.079 | 4.348 ± 0.181 |
| **$CCL_4$** | 389 ± 19.1 | 314.17 ± 11.71 | 31.24 ± 3.10 | 2.156 ± 0.047 | 72.870 ± 6.154 | 6.259 ± 0.066 | 3.880 ± 0.040 |
| **Sylimarin** | 309.5 ± 3.69* | 60.33 ± 2.10* | 14.86 ± 0.38* | 0.695 ± 0.019* | 54.069 ± 3.704* | 6.571 ± 0.057** | 4.561 ± 0.032** |
| **TME 100mg/kg** | 241.67 ± 7.013* | 345.33 ± 3.062** | 18.649 ± 4.62* | 1.120 ± 0.0362* | 59.211 ± 1.818* | 6.284 ± 0.085 | 4.258 ± 0.083** |
| **TME 200mg/kg** | 81.0 ± 4.487* | 297.83 ± 9.68* | 16.818 ± 1.831* | 0.941 ± 0.029* | 56.468 ± 3.181* | 6.606 ± 0.110** | 4.457 ± 0.0533** |
| ***F* calculated** | 14.896 | 313.46 | 6.261 | 239.38 | 6.233 | 4.126 | 7.556 |
| **Dunnet's value** | 112.11 | 94.54 | 84.12 | 1.04 | 35.70 | 1.82 | 0.30 |

Values are mean±S.E.M.; *F*theoretical = 2.79 ($P < 0.05$).
* Significant reduction compared to hepatotoxin ($P < 0.05$).
** Significant increase compared to hepatotoxin ($P < 0.05$).

Histopathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and a central vein (Fig. 1). Disarrangement of normal hepatic cells with intense centrilobular necrosis and vacuolization of periportal vein are observed in $CCl_4$-intoxicated liver (Fig. 2). The liver sections of the rat treated with methanolic extract (200 mg/kg) and intoxicated with CCl4 (Fig. 4), showed less vacuole formation and absence of necrosis and overall no visible changes observed as compared to silymarin (Fig. 3), supplementing the protective effect of the extract. Though the less visible changes are observed (Fig. 5) in the sections of the rats treated with methanolic extract (100 mg/kg) and intoxicated with $CCl_4$, their intensity was less compared to methanolic extract (200 mg/kg) treated rat sections.

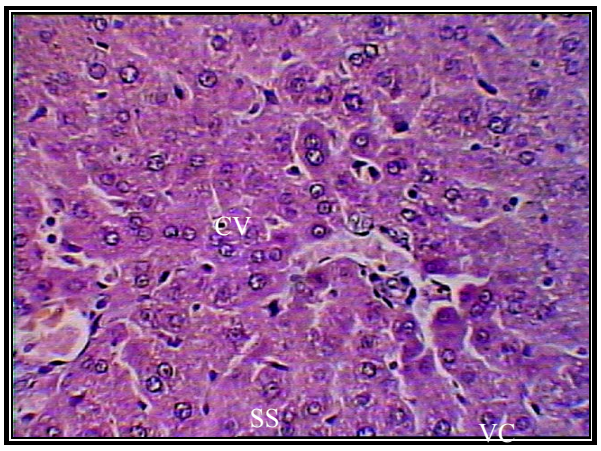


Fig.1. Normal rat liver section, 400×, haematoxylin–eosin stain. Liver section of the rat showing normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. CV: central vein; HC: hepatocyte; SS: sinusoidal space; VC: vacuole.

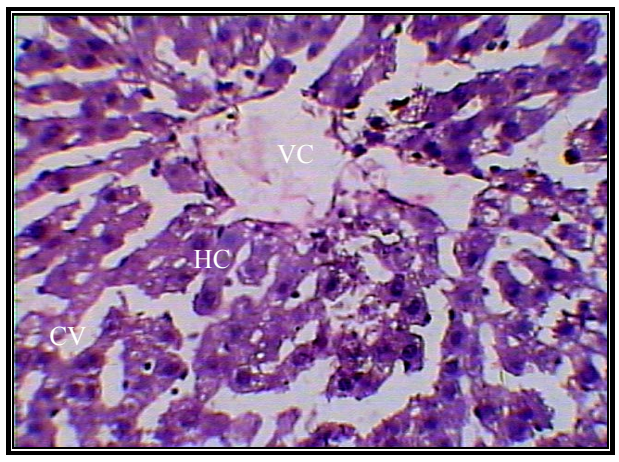


Fig.2. Liver section of rat intoxicated with CCl4, 400×, haematoxylin–eosin stain. Liver section of the rat showing disarrangement and degeneration of normal hepatic cells with with lobular necrosis, vacuol formation and fatty change. CV: central vein; HC: hepatocyte; SS: sinusoidal space; VC: vacuole.

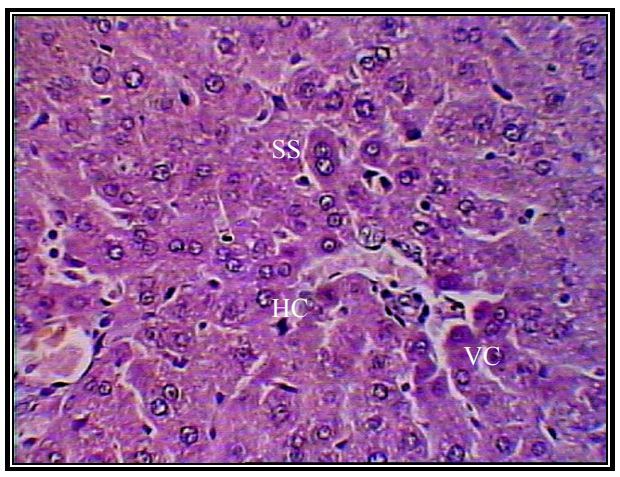


Fig.3. Liver section of rat treated with silymarin and intoxicated with CCl4. 400×, haematoxylin–eosin stain. Liver section of the rat shows less vacuole formation, reduced sinusoidal space and less fatty change compared to hepatotoxin. CV: central vein; HC: hepatocyte; SS: sinusoidal space; VC: vacuole.

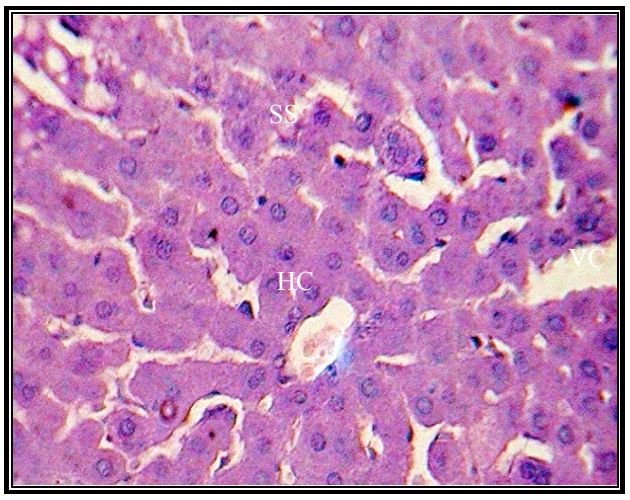


Fig.4. Liver section of rat treated with TME 100 mg/kg and intoxicated with CCl4, 400×, haematoxylin–eosin stain. Liver section of the rat shows less degeneration of hepatocyte, less vacuoles, disarrangement and fatty change compared to hepatotoxin. CV: central vein; HC: hepatocyte; SS: sinusoidal space; VC: vacuole.

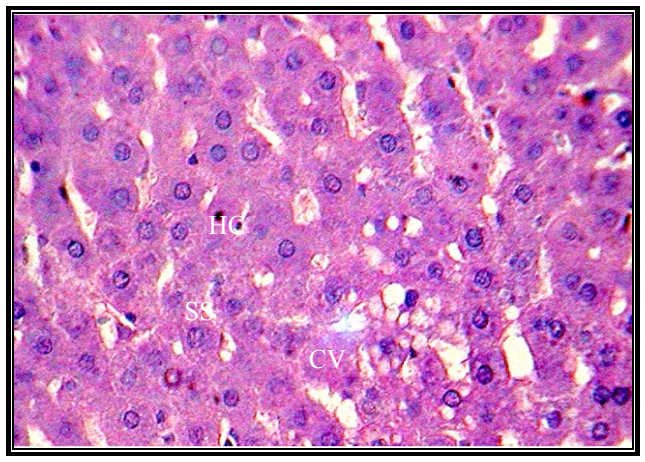


Fig.5. Liver section of rat treated with TME 200mg/kg and intoxicated with CCl4, 400×, haematoxylin–eosin stain. Liver section of the rat shows regeneration of hepatocyte, less vacuoles, disarrangement and fatty change compared to hepatotoxin. CV: central vein; HC: hepatocyte; SS: sinusoidal space; VC: vacuole.

## Discussion

In Indian system of medicine certain herbs are claimed to provide relief against liver disorders. The claimed therapeutic reputation has to be verified in a scientific manner. In the present study one such drug *Tecomella undulata* was taken for the study. The methanolic extract of *Tecomella undulata* possesses significant ($P < 0.05$) hepatoprotective effect in the $CCl_4$ model of intoxication in rats. Our investigation on the extracts showed the presence of triterpenoids and flavonoids in the methanolic extract. According to these results, it maybe hypothesized that flavonoids, which are present in the methanolic extract, could be considered responsible for the hepatoprotective activity.

The hepatotoxicity of $CCl_4$ has been reported to be due to the formation of the highly reactive trichloro free radical, which attacks polyunsaturated fatty acids. It produces Hepatotoxicity by altering liver microsomal membranes in experimental animals (36). The effect of $CCl_4$ is generally observed after 24 h of its administration. Hence the withdrawal of the blood for biochemical parameters should be carried out only after 24 h of $CCl_4$ intoxication. From Table 1 it is evident that the methanolic extract was able to reduce all the elevated biochemical parameters due to the hepatotoxin intoxication. The levels of total proteins and albumin were reduced due to the hepatotoxin intoxication. The reduction is attributed to the damage produced and localized in the endoplasmic reticulum which results in the loss of P450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides. Intoxication with $CCl_4$ also resulted in inhibition of synthesis of the bile acids from cholesterol which is synthesized in liver or derived from plasma lipids, leading to increase in cholesterol levels. Suppression of cholesterol levels suggests the inhibition of the synthesis of bile acids from cholesterol is reversed by the extract. Reduction in the levels of SGOT and SGPT towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by $CCl_4$. Reduction of ALKP levels with concurrent depletion of raised bilirubin level suggests the stability of the biliary function during injury with $CCl_4$. The raise in protein and albumin levels suggests the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by the methanolic extract is similar to silymarin treatment. The methanolic extract (100 mg/kg) was not able to reduce the elevated parameters caused by $CCl_4$ intoxication except the CHL. Similarly, an increase in the levels of ALB was observed with methanolic extract (100 mg/kg).

Histological examination of the liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. In the sections obtained from the rats treated with methanolic extract (200mg/kg) and intoxicated with hepatotoxin, the normal cellular architecture was retained as compared to silymarin, there by confirming the protective effect of the extract. Although the less visible changes are observed in the sections of the rats treated with methanolic extract (100 mg/kg) and intoxicated with $CCl_4$, the intensity was less compared to methanolic extract (200 mg/kg) treated rat sections.

It can be concluded from this investigation that, among the methanolic extracts (100 and 200 mg/kg) tested, the methanolic extract (200 mg/kg) of the bark parts of *Tecomella undulata* possess hepatoprotective activity against $CCl_4$ intoxication in rats. Our further detailed studies may, however, confirm the utility profile of this drug.

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