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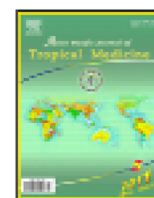

Document heading doi:

# Hepatoprotective activity of *Terminalia paniculata* against paracetamol induced hepatocellular damage in Wistar albino rats

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ARTICLE INFO

*Article history:*
Received 23 February 2011
Received in revised form 27 March 2011
Accepted 15 April 2011
Available online 20 June 2011

*Keywords:*
*Terminalia paniculata*
Hepatoprotection
Paracetamol
Liv 52
Silymarin

ABSTRACT

**Objective:** To evaluate the hepatoprotective activity of *Terminalia paniculata* against paracetamol induced hepatic damage in rats. **Methods:** The plant material was shade dried, powdered and extracted with ethanol. Liv 52 and silymarin were used as standard drugs and 2% gum acacia as a control (vehicle). Alteration in the levels of biochemical markers of hepatic damage like AST, ALT, ALP and lipid peroxides were tested, and phytochemical tests were also performed. **Results:** Paracetamol (2 g/kg) increased the serum levels of alanine aminotransfer (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and the lipid peroxides. Treatment of Liv 52, silymarin and ethanolic extract of *Terminalia paniculata* (200 mg/kg) altered levels of biochemical marker and showed significant hepatoprotective activity. Ethanolic extract revealed the presence of phenolic compound and flavanoids. Our findings suggested that ethanolic bark extract of *Terminalia paniculata* possessed hepatoprotective activity in a dose dependent manner. **Conclusions:** *Terminalia paniculata* possesses hepatoprotective activity. It could be an effective and promising preventive agent against PCT induced hepatotoxicity.

## 1. Introduction

Liver, the key organ of metabolism and excretion, possesses the function of detoxifying xenobiotics, environmental pollutants and chemotherapeutic agents. Liver diseases are the most serious ailment and are mainly caused by toxic chemicals (excess consumption of alcohol, high doses of paracetamol, carbon tetrachloride, chemotherapeutic agents, peroxidised oil, *etc*)[1].

Acetaminophen also known as paracetamol (PCM) is commonly used as effective antipyretic and analgesic agent for relieving mild and moderate pain. However, either accidental or deliberate overdose can cause hepatotoxicity[2]. Acetaminophen induced hepatotoxicity is currently the most important cause for acute hepatic damage and death in US[3].

Now the mechanism of this injury is still not entirely clear. Key features of the toxic mechanism include the formation of a reactive metabolite, presumably *N* acetyl-p-benzoquinone imine (NAPQI), which is quickly conjugated by hepatic glutathione to yield a harmless product called mercapturic acid. However, due to overdose of acetaminophen, the capacity of glucuronidation and sulfation is exceeded with the formation of excess NAPQI via cytochrome P-450 2E1. After glutathione is depleted, excessive NAPQI binds to hepatic cell proteins and DNA which precedes liver injury[4]. Liver damage is associated with cellular necrosis, increase in lipid peroxidation and depletion in glutathione (GSH) level. In addition, serum levels of many biochemical markers like aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglycerides, cholesterol, bilirubin, and lactate dehydrogenase (LDH) are elevated[1]. Conventional hepatoprotective drugs used for the treatment of such adverse reactions are often inadequate and it is needed to dechallenge the offending drug.

Therefore, it is important to explore hepatoprotective effect of natural products. In spite of the tremendous advances in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. In India, about 40 polyherbal commercial formulations with hepatoprotective action are being used. It has been reported that 160 phytoconstituents

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from 101 plants have hepatoprotective activity[5]. Hepatoprotective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines. Many extracts of plants are also used for the treatment of liver disorders. About 25 plants have been reported in treatment of liver disorders[5]. Therefore, it is essential to explore new medicinal plants and to develop more effective and cheaper drugs.

Very few drugs can stimulate liver function, protect the liver from damage or promote the regeneration of hepatic cell. However, many formulations containing herbal extracts are employed in traditional system of medicine for liver diseases in India[5]. Some of these plants have already been reported to possess strong antioxidant activity[5, 6].

*Terminalia paniculata (T. paniculata)* Roth. (Combretaceae) is a tropical tree with a large natural distribution in Western Ghats, India. Traditionally, flower juice and bark of *T. paniculata* have been used in the treatment for cholera, inflammation of parotid glands and menstrual disorders[7]. It is also used to treat cough, bronchitis, cardiac debility, diabetes, wounds and skin diseases. But still no investigation has so far been reported on its hepatoprotective activity. This study is to explore to the possible mechanism of hepatoprotective activity of ethanolic extract of *T. paniculata*. The ethanolic extract is used in this investigation because ethanol is more nonpolar than water. The major active chemical constituents of *T. paniculata*, including volatile oils would be more soluble in ethanol fraction than in water extract.

## 2. Materials and methods

### 2.1. Selection of animals, caring and handling

This was done as per the guidelines of committee for the purpose of control and supervision of experiments on animals. A total of 42 healthy albino Wistar rats (150-200 g), aged twelve weeks of either sex, bred in the animal house of Kasturba Medical College, Manipal were selected. They were kept under temperature of (23±2) ℃, humidity of 50% and 10 h:14 h of light and dark cycles, respectively. They were housed individually in polypropylene cages containing sterile paddy husk (procured locally) as bedding throughout the experiment and had free access to sterile food and water *ad libitum*. Animals were fasting overnight and weighed before the experiment. The study was under the approval of Institutional Animal Ethics Committee (IAEC No. IAEC/KMC/07/2008-2009 Dated September.06.2008).

### 2.2. Reagents and instruments

Paracetamol 2 g/kg (Themis Pharmaceuticals, Chemoxyn Pvt. Ltd.), Liv 52 1 mL/rat/day (Himalaya Pvt. Ltd.), silymarin 25 mg/kg bw (Sigma Aldrich-USA), alcoholic extract of *T. paniculata*, 2% gum acacia (E. Merck India Pvt Ltd.), Cobas Fullyautomated autoanalyser, LFT Cobas Kits.

### 2.3. Collection and preparation of ethanolic extract of T. paniculata

*T. paniculata* plants were procured locally in August 2009 and authenticated by the professor of Botany, Mahatma Gandhi Memorial College, and Udupi. The shade dried bark were crushed into small pieces and powdered. The powder was loaded into Soxhlet extractor with glass thimble (cat No.3485) in 8 batches of 250 g each and was subjected to extraction for about 30-40 h with 95% ethanol. After extraction the solvent was distilled and the extract was concentrated under reduced pressure on a water bath at a temperature below 50 ℃ to a syrupy consistency. Then it was dried in the desiccators. The yield was about 8%.

### 2.4. Acute toxicity test

Doses were selected and determined according to the acute toxicity test reported previously[8]. The dose of 3 g/kg was well tolerated without any signs of toxicity and mortality. So we presumed that $LD_{50}$ was beyond the dose of 3 g/kg bw. Three different graded doses 100 mg/kg, 200 mg/kg and 400 mg/kg bw, were selected for test of hepatoprotective activity.

### 2.5. Study design and experimental protocol

The rats were randomly divided into seven groups of six rats. Rats in Group Ⅰ (Control) received 2 mL of 2% gum acacia po through intragastric tube for 8 days. Group Ⅱ were treated by 2% gum acacia + PCM (2 g/kg) po, Group Ⅲ by Liv 52 (1 mL/rat/day) po, Group Ⅳ by silymarin (25 mg/kg) ip, while Group Ⅴ, Ⅵ and Ⅶ received ethanolic plant extract po at 100, 200 and 400 mg/kg bw through intragastric tube, respectively.

Plant extract, paracetamol were administered orally by suspending in 2 mL of 2% gum acacia through intragastric tube. The standard hepatoprotective drugs, silymarin and Liv 52 were given orally and intraperitoneally, respectively. All reagents were administered daily for 7 consecutive days. And on the 8th day single dose of paracetamol 2 g/kg was given to all group rats except rats in group Ⅰ.

On the 9th day, after 24 hours of paracetamol administration blood samples were collected by direct cardiac puncture under light ether anesthesia. Serum was separated by centrifuging at 2 500 rpm for 15 min and used for analysis of various biochemical parameters, including AST, alanine aminotranferase (ALT), ALP, total proteins (TP) and albumin (ALB). All rats were sacrificed by cervical dislocation, and livers were removed. The weight and volume of the liver were measured immediately. Livers were washed with ice-cold saline and a 50% homogenate prepared in 0.05 M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 700 × *g* for 10 minutes at 4 ℃ and the

supernatant was used for the estimation of malondialdehyde (MDA), the end product of lipid peroxidation and GSH.

### *2.6. Statistical analysis*

Data were analysed using SPSS (versus 16) package. Results were expressed as Mean±SEM. The total variation was analyzed by one way analysis of variance (ANOVA). Differences among mean values were analyzed by Dunnet's post hoc test with *P* value < 0.05 significant.

## 3. Results

PCM treated rats showed significant increase in the serum levels of ALT, AST, LDH, ALP, total bilirubin (TB), and lipid peroxidation (LPO) and decrease in serum levels of TP, ALB and total thiols (TH) ($P$<0.05) (Table 1).

The treatment with silymarin and Liv 52 significantly reduced the levels of ALT, AST, LDH, ALP, TB and LPO ($P$<0.05), and increased the levels of TP, ALB and TH($P$<0.05). While extract of *T. paniculata* plant also showed significant hepatoprotective activity ($P$<0.05). The 200 mg/kg of plant extract was more effective on the levels of ALT, AST, LDH, ALP and TB than other two dosage groups (Table 1).

PCM (2 g/kg po.) led to extensive necrosis and haemorrhages in hepatic tissues. Low dose of *T. paniculata* produced partial recovery and with higher dose, there was near complete without punctate haemorrhages, swelling and with smooth margins. The mean weight of the liver was decreased in PCM treated group significantly [(3.51±0.11) g per cent of body weight] ($P$<0.05). While three doses of plant extract increased mean weight and volume of the significantly($P$<0.05). These increases were comparable to standard silymarin and Liv 52 treated groups (Table 1).

**Table 1**

Hepatoprotective activity of ethanolic extract of *T. paniculata* (Mean ±SEM).

| Indexes | Control | PCM Group | Silymarin Group | Liv 52 Group | 100 mg/kg *T. paniculata Group* | 200 mg/kg *T. paniculata Group* | 400 mg/kg *T. paniculata Group* |
|---|---|---|---|---|---|---|---|
| TB(mg/dL) | 0.41±0.03 | 1.98±0.26 [a] | 1.09±0.22 [b] | 0.90±0.10 [b] | 0.81±0.15 [b] | 0.39±0.06 [bc] | 0.65±0.27 [b] |
| ALP(IU/L) | 64.90±1.79 | 187.24±3.12 [a] | 118.55±3.13 [b] | 103.70±1.56 [b] | 102.79±1.78 [bc] | 78.11±1.25 [bcd] | 93.57±1.57 [bcd] |
| AST(IU/L) | 49.05±2.24 | 162.62±2.68 [a] | 108.93±0.94 [b] | 99.32±0.90 [b] | 103.31±0.91 [b] | 84.54±0.85 [bcd] | 101.06±0.92 [bc] |
| ALT(IU/L) | 35.67±2.61 | 112.68±1.23 [a] | 81.30±0.52 [b] | 55.18±0.77 [b] | 48.29±0.71 [bcd] | 41.67±0.54 [bcd] | 44.84±0.61 [bcd] |
| LDH(IU/L) | 105.09±2.80 | 356.53±3.53 [a] | 194.10±2.47 [b] | 163.94±1.13 [b] | 155.43±1.04 [bcd] | 150.30±0.71 [bcd] | 166.64±1.15 [bc] |
| ALB(g/L) | 4.56±0.25 | 1.68±0.20 [a] | 2.68±0.08 [b] | 2.84±0.14 [b] | 2.49±0.21 [b] | 3.99±0.11 [bcd] | 3.67±0.14 [bcd] |
| Total thiols (IU) | 178.87±3.37 | 26.51±1.31 [a] | 58.18±1.60 [b] | 78.61±1.72 [b] | 99.09±1.59 [bc] | 139.22±3.68 [bcd] | 79.50±2.40 [bcd] |
| LPO(nmol/min/L) | 0.14±0.08 | 3.17±0.13 [a] | 2.83±0.14 | 2.81±0.15 | 2.61±0.23 | 1.56±0.11 [bcd] | 1.91±0.22 [bcd] |
| TP(mg/L) | 94.71±2.35 | 17.85±0.98 [a] | 33.25±1.68 [b] | 51.68±1.95 [b] | 66.40±1.28 [bc] | 81.7±1.12 [bcd] | 78.15±1.80 [bcd] |
| Liver volume(mL) | 4.16±0.16 | 3.51±0.11 [a] | 5.78±0.84 [b] | 5.04±0.84 [b] | 4.06±0.22 b | 5.02±0.24[b] | 5.83±0.06 [b] |
| Liver weight(g) | 4.07±0.12 | 3.15±0.07[a] | 5.38±0.20 [b] | 5.68±0.24 [b] | 4.90±0.06 b | 5.64±0.42[bd] | 5.20±0.06 [bcd] |

[a]=$P$<0.05 compared with control, [b]=$P$<0.05 compared with paracetamol, [c]= $P$<0.05 compared with Silymarin, [d]=$P$<0.05 compared with Liv 52.

## 4. Discussion

Paracetamol is a known antipyretic and an analgesic which produces hepatic necrosis in high doses. It is usually eliminated mainly as sulfate and glucuronide. At toxic doses, the sulfation and glucuronidation routes become saturated and hence, higher percentages of paracetamol molecules are oxidized to highly reactive NAPQI by cytochrome-450 enzymes. Semiquinone radicals, obtained by one electron reduction of NAPQI, can covalently bind to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage. Higher doses of paracetamol and NAPQI can produce alkylate and oxidise intracellular GSH, which results in the depletion of liver GSH pool, and subsequently leads to increased lipid peroxidation and liver damage[9-16].

In the assessment of liver damage by acetaminophen the determination of enzyme levels such as AST, ALT, ALP and LDH is most commonly used. Hepatocellular necrosis or membrane damage releases the enzymes into circulation and hence it can be measured in the serum. High level of AST indicates liver damage, such as viral hepatitis, cardiac infarction and muscle injury. AST catalyses the conversion of alanine to pyruvate and glutamate, and is released in a similar manner. Therefore, ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. On other hand, serum levels of ALP, bilirubin and TP are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in the presence of increasing biliary pressure[17].

Results of our study showed that acetaminophen causes a significant elevated levels of AST, ALT, ALP, LDH, TB, LPO and decrease in TP and ALB ($P$<0.01). After administration of the plant extract, Liv 52 or silymarin, the levels of these enzymes were restored in a dose-dependent manner. The reversal may be because of the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes[18]. Effective control of ALP, bilirubin and total

protein levels point towards an early improvement in the secretory mechanism of the hepatic cells. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. The drugs like Liv 52, silymarin and the plant extract decreased elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells.

Acetaminophen induces increase in LPO and decrease in total serum protein (TSP). This suggests lipid peroxidation can lead to tissue damage and failure of antioxidant defense. *T. paniculata* extract significantly reversed these changes. Hence, its potent antioxidant property is likely one of the mechanisms of hepatoprotection.

*T. paniculata* can affect the glutathione of the blood and liver cells. It significantly increased the hepatic and blood thiols. The results suggest that the higher content of glutathione in blood and liver would offer a better protection against an oxidative stress, thus contributing to the abolishment of paracetamol induced hepatotoxicity.
A reduction in TSP andf ALB may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize proteins and consequently decrease in the liver weight. But, when *T. paniculata* extract, silymarin and Liv 52 were given, there was significant increase in TSP and ALB indicating the hepatoprotection activity and also accounting for the increase in the liver weight most probably through the hepatic cell regeneration.

Free radical mediated process has been implicated in pathogenesis of most diseases. The protective effect of *T. paniculata* on acetaminophen induced hepatotoxicity in rats appears to be related to inhibition of lipid peroxidation and increased levels of antioxidant enzyme in addition to free radicals scavenging action. Preliminary phytochemical studies reveal the presence of flavonoids in ethanolic extract of *T. paniculata*. Flavonoids are hepatoprotectives[16]. The observed antioxidant and hepatoprotective activity of *T. paniculata* may be attributed to the presence of flavonoids. Further studies to characterise the active principles and to elucidate the mechanism are in progress.

The ethanolic bark extract of *T. paniculata* has shown the ability to maintain the normal functional status of the liver. From the above preliminary study, we conclude that the ethanolic extract of *T. paniculata*, is proved to be one of the herbal remedies for liver ailment especially for PCM induced hepatotoxicity.

## Conflict of interest statement

We declare that we have no conflict of interest.

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