Hepatoprotective Potential of *Trichosanthes dioica* Roxb in Hepatotoxicity Induced by Simvastatin and its Consequences on Biochemical and Haematological Indices

Ramesh Kumar Gupta\(^1,2,6^*\), Sudhansu Ranjan Swain\(^3\), Jagannath Sahoo\(^4\), Amresh Gupta\(^5\), Sachin Chaudhary\(^6\)

\(^{1}\)Sherwood College of Faculty of Pharmacy, Barabanki -225001, Uttar Pradesh, INDIA.
\(^{2}\)S.R.M.S. College of Engineering and Technology, Bareilly-243202, Uttar Pradesh, INDIA.
\(^{3}\)Moradabad Educational Trust Group of Institutions, Faculty of Pharmacy, Moradabad-244001, Uttar Pradesh, INDIA.
\(^{4}\)KIET School of Pharmacy, Ghaziabad-201001, Uttar Pradesh, INDIA.
\(^{5}\)Goel Institute of Pharmacy and Sciences, Lucknow-226024, Uttar Pradesh, INDIA.
\(^{6}\)College of Pharmacy, University of Sharjah, Sharjah-27272, UNITED ARAB EMIRATES.

**Correspondence**
Ramesh Kumar Gupta
Sherwood College of Faculty of Pharmacy, Barabanki -225001, Uttar Pradesh, INDIA.
Phone no : 9451529612
E-mail: ram5880@gmail.com

**ABSTRACT**

**Objective:** To evaluate hepatoprotective activity along with hematological and defensive recital of *Trichosanthes dioica* Roxb against simvastatin induced hepatotoxicity in experimental rodents.

**Methods:** In the present study, *in-vivo* hepatoprotective effect of 50% methanolic fruit extract of *Trichosanthes dioica* Roxb (TME 200 and 400 mg/kg body weight) was evaluated using experimental model, simvastatin (20 mg/kg, p.o.), induced hepatotoxicity in experimental animals. The hepatoprotective activity was estimated by interpreting using various biochemical parameters like SGOT, SGPT, ALP, total bilirubin, total protein and albumin along with the haematological and histopathological studies. **Results:** The treatment with TME significantly (P<0.05-P<0.001) and dose-dependently reversed simvastatin induced elevation in serum level of SGOT, SGPT, ALP, total bilirubin and restored the total protein and albumin level. Furthermore, TME also signify the blood parameters at dose of 1000 and 2000 mg/kg and restored the body defense mechanism. The histological examination revealed that TME at dose of 200 mg/kg showed regeneration of hepatocytes around central vein with near normal liver architecture. **Conclusion:** The results of this study exhibited liver protective effect of *Trichosanthes dioica* Roxb against simvastatin induced liver injury and they may be support its traditional use.

**Key words:** Hepatocytes, Hepatotoxicity, Liver, Simvastatin, *Trichosanthes dioica*.

**INTRODUCTION**

*Trichosanthes dioica* Roxb, commonly known as *parwal*, belongs to cucurbitaceae family and is an annual or perennial herb distributed throughout India. Out of 20 species in India, two are cultivated as vegetable (*T. anguina* and *T. dioica*). In Charaka Samhita, leaves and fruits are enlisted for treatment of alcoholism and jaundice. In Ayurveda, leaves are utilized as antipyretic, diuretic, cardiotonic, laxative, antiulcer. *T. dioica* accommodate numerous chemical constituents like vitamin A, saponins, tannins, alkaloids, mixture of novel peptides, proteins and vitamin C.\(^1,2\) Numerous scientific research revealed that *T. dioica* exhibited anti-diabetic,\(^3\) anti-hypercholesteraeimic,\(^4\) hepatoprotective,\(^5\) anti-ulcer,\(^6\) immunomodulatory,\(^7\) antimicrobial,\(^8\) anti-oxidant,\(^9\) anti-diarrheal,\(^10\) nephroprotective,\(^11\) and wound healing activity.\(^12\) HMG CoA reductase inhibitors (Simvastatin) are effective in reducing cardiovascular mortality and are widely prescribed around the globe. More than 145 million patients were prescribed with statins in United States in 2005. The use of statins is increasing day by day, although liver toxicity has been a concern since their initial introduction.\(^13\) No scientific results are available affirming hepatoprotective potential of *Trichosanthes dioica* Roxb against hepatotoxicity induced by simvastatin. Hence, present investigation was designed to demonstrate hepatoprotective activity of *Trichosanthes dioica* Roxb against simvastatin induced liver toxicity.

**MATERIALS AND METHODS**

**Drugs and Chemicals**

Simvastatin (Merck Pharmaceutical, India). All chemicals used were of analytical grade and procured from Sigma Chemicals Co., USA and Qualigens fine chemicals, Mumbai, India.

**Collection and authentication of plant**

Fresh and matured fruits of *T. dioica* were purchased from local market of Lucknow, India in August 2016. The plant material was identified and authenticated by National Botanical Research Institute, Lucknow, India.

**Extraction of plant material**

The fruits of *T. dioica* were dried and powdered. The powdered plant material was macerated with petroleum ether; the marc was exhaustively extracted with 50% methanol for three days. The extract was dried by rotator evaporator (IK, Germany) under reduced pressure and procured in
desiccator. The % yield was discovered to be 0.75%, 1% Tween-80 was used to prepare extract suspension of desirable concentration needed for pharmacological studies.

**Phytochemical investigation**

The methanolic extract of *T. dioica* fruits were subjected to preliminary phytochemical screening for detecting the presence or absence of active phytochemical constituents.14,15

**Animals**

Wistar rats weighing (150-170 g) of either sex were procured from Animal house of College of Pharmacy, Shri Ram Murti Smarak College of Engineering and Technology, Bareilly, India. They were kept in departmental animal house in well cross ventilated room at 22±2°C with light and dark cycles of 12 h for 1 week before and during the experiments. The experimental protocols were approved by Institutional Animal Ethical Committee, India (Reg. No. 715/02/CPCSEA).

**Acute toxicity study**

Acute toxicity study was performed according to OECD guidelines 423. Albino mice (20-25 g) were divided into five groups with 5 mice in each. Group-I numbered as control received distilled water orally. Group-II, III, IV, and V were administered *T. dioica* extract at a dose of 5, 50, 300, 2000 mg/kg, orally, respectively. The animals were noticed for toxicity sign or mortality every 24 h, daily for 2 weeks.16

**Experimental design**

Wistar rats were divided into five different groups, each group having 6 rats. Group I received distilled water only for 30 days. Group II rats charged with simvastatin (20 mg/kg, p.o.) alone for 30 days orally. Group III and IV rats received simvastatin along with *T. dioica* fruits extracts (200 mg/kg and 400 mg/kg, p.o.) respectively for 30 days and Group V rats received simvastatin along with silymarin (20 mg/kg,p.o.) for 30 days. On the 31st day, blood samples were collected, and all the animals were sacrificed by cervical dislocation under mild ether anesthesia and liver sample were harvested, rinsed in saline and stored at -80°C for further biochemical analysis.17

**Evaluation of liver protective activity**

The collected blood was allowed to clot and serum was separated by centrifugation in a refrigerator tabletop centrifuge at 2500 rpm for 15 min and the biochemical parameters like serum enzymes: Serum glutamic oxaloacetic transaminase (SGOT, U/L), serum glutamic-pyruvic transaminase (SGPT, U/L),19 alkaline phosphatase (ALP, U/L),20 total bilirubin (mg/dL),20 total protein and albumin were evaluated.21,22

**Evaluation of hematological parameter**

Red blood cell (RBC) count, haemoglobin (Hb), white blood cell (WBC) count, platelet (PLT) and lymphocytes were determined by the fully automated hematology analyzer (XP 100 Hematology Analyzer, Transasia Bio-Medicals Ltd., India).

**Histopathological studies**

For histopathological inspection, the liver tissues were affixed with 10% phosphate buffer neutral formalin, dehydrated in graded (50-100%) alcohol and embedded in paraffin. fine sections (5 μm) were cut and stained with routine haematoxylin and eosin stain for photo microscopic analysis. All the slides were studied under a light microscope for any histological destruction and protection.

**Statistical analysis**

The statistical comparison between the groups were made by One Way Analysis of Variance (ANOVA) and followed by Student-Newman-Keuls test. The value *p*<0.05 was considered statistically using, Graph Pad Prism 5.03 Software. The values were represented as mean ± SEM for six rats.

**RESULTS**

**Phytochemical screening**

Phytochemical screening showed the presence of tannins, alkaloids, carbohydrates, flavonoids, glycosides and steroids as documented in Table 1.

**Acute toxicological outcome**

The methanolic extract of *T. dioica* at doses of 200 and 400 mg/kg body weight does not produce any toxic outcome. Therefore, these doses were selected for hepatoprotective studies.

**Effect of TME on serum hepatic parameters**

The outcome of *T. dioica* fruits extract dose was investigated on liver serum markers like SGOT, SGPT, ALP, bilirubin (BLB), total protein (TP) and albumin (ALB) level. Hepatic abrasion due to dose of simvastatin generate significant elevation in marker enzymes as SGOT by 336.5%, SGPT by 135.7%, ALP by 144%, BLB by 39%, and demotion in level of TP by 48.3% and ALB by 32.5% when compared to control (Group I). The dose of extract, TME 200 (Group III) and TME 400 (Group IV) declined the elevated level of SGOT (10.19%, *P*<0.01, 66.3%, *P*<0.001), SGPT (15.41%, *P*<0.01, 73.2%, *P*<0.001), ALP (7.30%, *P*<0.05, 29.68%, *P*<0.001), BLB (12.64%, *P*<0.01, 53.12%, *P*<0.001) and promote the level of TP (17.94%, ns, 37.25%, *P*<0.01, ALB (21.61%, *P*<0.01, 26.36%, *P*<0.001) respectively as compared to group II. Similarly, silymarin decreased SGOT by (120.6%, *P*<0.001), SGPT (110.1%, *P*<0.01, ALP (105%, *P*<0.001), BLB (216%, *P*<0.001) and increased TP by (42.85%, *P*<0.001), ALB (30.57, *P*<0.001) as compared to group II. The results are tabulated in Table 2.

**Effect of TME on body weight and liver weight**

The effect of different doses of TME on body weight and liver weight were studied (Table 3). In Group II body weight decreased by 5.845% while liver weight increased by 26.36%. Animal Treated with TME at the doses of 200 and 400 mg/kg (Group III and IV) significantly increased in body weight by 2.15%, 3.81% respectively while liver weight was decreased by 1.01% and 8.56% respectively.

**Effect of TME on blood parameters**

The outcome in (Table 4) showed a significant change in RBC, Hb, PLT, WBC, and % Lymphocytes counts. RBC PLT, WBC, and % Lymphocytes counts significantly increased at the dose of 1000 and 2000 mg/kg, while Hb count non-significantly increases at the dose of 1000 mg/kg as compared to control group. Hb, PLT, WBC and % Lymphocytes counts significantly increased at the dose of 4000 mg/kg, whereas RBC’s count non-significantly increases at the dose of 4000 mg/kg when compared to the control group.

**Histopathological observations**

The histological explanation (Figure 1) support the results obtained from serum enzyme assays. Liver sections of control rats showed normal hepatic cells with well-preserved cytoplasm and well brought out central vein. Simvastatin (20mg/kg) treated rats (Group II), displayed the massive fatty changes, necrosis, central vein congestion, ballooning degeneration, and the loss of cellular boundaries, whereas TME 200mg/kg treated groups (Group III) showed mild congestion in central vein with less fatty changes, mild necrotic cells, with minimal inflammatory conditions and less infiltration of the leucocyte’s while TME 400mg/kg treated group (Group IV) showed regeneration of hepatocyte around central vein with near normal liver architecture, prominent nucleus and possessing maximum hepatoprotective action. Rats in (Group V) exhibited well
brought out central vein, hepatic cell with well-preserved cytoplasm, prominent nucleus.

DISCUSSION

The WHO survey confirmed that 70-80% of the world population rely on noncommercial medicine from herbal sources in primary health care units.\textsuperscript{23} The results of the present study clearly indicated hepatoprotective effects of the methanolic fruits extract of \textit{Tricoxanthes dioica} against simvastatin induced liver toxicity in rats. Liver is a vital organ within the body, playing essential role in metabolic homeostasis and detoxification of variety of drugs and xenobiotic.\textsuperscript{24} Assessment of liver function can be performed by estimating the activities of serum SGOT, SGPT, ALP, BLB, TP and ALB. 

### Table 1: Preliminary phytochemical analysis of \textit{T. dioica} fruit extract.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of test</th>
<th>Constituents</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ferric Chloride &amp; Gelatin test.</td>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Mayers&amp;Wagners Test.</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Benedict’s test.</td>
<td>Carbohydrates</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Ferric Chloride &amp; Shinoda Test.</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Keller Killiani &amp; Bromine water test.</td>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Salkowski Test.</td>
<td>Steroids</td>
<td>+</td>
</tr>
</tbody>
</table>

“+” means present.

### Table 2: Effect of TME on serum SGOT, SGPT, ALP, BLB, TP and ALB against simvastatin induced liver toxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT</th>
<th>SGPT</th>
<th>ALP</th>
<th>BLB</th>
<th>TP</th>
<th>ALB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.2 ± 1.5</td>
<td>41.3 ± 2.1</td>
<td>87.32 ± 3.2</td>
<td>0.2 ± 0.01</td>
<td>6.2 ± 0.6</td>
<td>3.6 ± 0.01</td>
</tr>
<tr>
<td>SIM</td>
<td>136.2 ± 4.3\textsuperscript{†}</td>
<td>97.35 ± 3.4\textsuperscript{†}</td>
<td>213.1 ± 4.2\textsuperscript{†}</td>
<td>0.98 ± 0.02\textsuperscript{†}</td>
<td>3.2 ± 0.4\textsuperscript{†}</td>
<td>2.43± 0.02\textsuperscript{†}</td>
</tr>
<tr>
<td>TME200</td>
<td>123.6 ± 3.4\textsuperscript{b}</td>
<td>84.35 ± 3.1\textsuperscript{b}</td>
<td>198.6 ± 4.1\textsuperscript{b}</td>
<td>0.87± 0.04\textsuperscript{b}</td>
<td>3.9 ±0.3\textsuperscript{c}</td>
<td>3.1±0.1\textsuperscript{c}</td>
</tr>
<tr>
<td>TME400</td>
<td>81.9 ± 3.1\textsuperscript{a}</td>
<td>56.2 ± 2.9\textsuperscript{a}</td>
<td>164.32 ± 3.2\textsuperscript{a}</td>
<td>0.64 ± 0.03\textsuperscript{a}</td>
<td>5.1 ± 0.2\textsuperscript{a}</td>
<td>3.3 ± 0.02\textsuperscript{a}</td>
</tr>
<tr>
<td>SYL 20</td>
<td>61.74 ± 2.9\textsuperscript{c}</td>
<td>46.32 ± 2.1\textsuperscript{c}</td>
<td>103.5 ± 3.6\textsuperscript{c}</td>
<td>0.31 ± 0.02\textsuperscript{c}</td>
<td>5.6 ± 0.4\textsuperscript{c}</td>
<td>3.5 ± 0.02\textsuperscript{c}</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. of 6 rats in each group, ns: non-significant.

P values: \(†<0.001\) compared with respective control group I.

P values: \(<0.05, \<0.01, \<0.001\) compared with group II.

### Table 3: Effect of TME on body weight and liver weight against simvastatin induced liver toxicity in rats.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>172.8±1.2</td>
<td>6.42±0.01</td>
</tr>
<tr>
<td>2</td>
<td>SIM</td>
<td>162.7±1.3\textsuperscript{c}</td>
<td>7.92±0.02\textsuperscript{c}</td>
</tr>
<tr>
<td>3</td>
<td>TME200</td>
<td>166.2±1.3\textsuperscript{a}</td>
<td>7.84±0.03\textsuperscript{a}</td>
</tr>
<tr>
<td>4</td>
<td>TME400</td>
<td>168.9±1.4\textsuperscript{b}</td>
<td>7.24±0.02\textsuperscript{b}</td>
</tr>
<tr>
<td>5</td>
<td>SYL 20</td>
<td>170.8±1.1\textsuperscript{c}</td>
<td>6.98±0.01\textsuperscript{c}</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. of 6 rats in each group, ns: non-significant.

P values: \(<0.05, \<0.01, \<0.001\) compared with group II.

### Table 4: Effect of TME on blood parameters against simvastatin induced liver toxicity in rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>1000 mg/kg</th>
<th>2000 mg/kg</th>
<th>4000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (x10\textsuperscript{12})</td>
<td>7.6 ± 0.1</td>
<td>8.1 ± 0.12\textsuperscript{a}</td>
<td>8.4 ± 0.13\textsuperscript{c}</td>
<td>7.9 ± 0.16\textsuperscript{a}</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.01 ± 0.2</td>
<td>13.91 ± 0.3\textsuperscript{a}</td>
<td>14.6 ± 0.4\textsuperscript{a}</td>
<td>14.4 ± 0.5\textsuperscript{a}</td>
</tr>
<tr>
<td>PLT (x10\textsuperscript{9}/L)</td>
<td>613.2 ± 5.6</td>
<td>634.6 ± 6.2\textsuperscript{a}</td>
<td>645.2 ± 4.6\textsuperscript{a}</td>
<td>653.5 ± 5.1\textsuperscript{a}</td>
</tr>
<tr>
<td>WBC (x10\textsuperscript{9})</td>
<td>8.1 ± 0.12</td>
<td>9.2 ± 0.2\textsuperscript{a}</td>
<td>11.5 ± 0.6\textsuperscript{a}</td>
<td>10.01 ± 0.3\textsuperscript{c}</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>41.2 ± 2.1</td>
<td>50.3 ± 3.2\textsuperscript{a}</td>
<td>54.2 ± 3.1\textsuperscript{c}</td>
<td>58.6 ± 3.5\textsuperscript{c}</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M. of 6 rats in each group, ns: non-significant.

P values: \(<0.001\) compared with respective control group I.

P values: \(<0.05, \<0.01, \<0.001\) compared with group II.
ALP, total bilirubin, albumin and total proteins, which are originally present in higher concentrations in hepatocytes. During liver disease, these enzymes leak into the bloodstream in conformity with the extent of liver damage.26 Bilirubin is an index of liver function and its elevated level indicates damage to the liver and bile duct.27 Liver damage induced by simvastatin represents disturbances of metabolism of liver cells that leads to distinctive changes in the liver serum markers. The increased levels of hepatic serum markers like SGOT, SGPT, ALP, total bilirubin and decreased in the albumin, was observed in simvastatin treated animals; this may be due to the changes in the cell membrane permeability indicating severity of hepatocellular damage.27,28 The animals treated with methanolic extract of T. dioica significantly reduced the levels of SGOT, SGPT, ALP, total bilirubin while increase total protein and albumin levels in dose dependent manner as compared with simvastatin as well as silymarin treated animals. Blood parameters dispense valuable data regarding health of animals.29

Administration of the plant extract resulted in significant increment in Hb, PLT, WBC and % Lymphocytes counts at the dose of 4000 mg/kg. RBC and Hb are vital in transporting respiratory gases. The increase in levels of RBC and Hb implies that extract did not adversely affect oxygen carrying capacity of the blood and the amount of oxygen delivered to tissues, therefore can be used in anaemia.30 The significant increment in the platelet count following administration of plant extract is the indication of stimulation of thrombopoietin creation as it has hemostatic capability of the blood and upholding blood clotting mechanism.31 Inflammatory response is characterized by the involvement of WBC. In this study, the incrementin level of WBC indicates the stimulation of immune system, in retort to toxic environment.32 Lymphocytes are the key cells of the immune system and elevation in its level indicates pathogenic attack and play the chief role in body defense mechanisms.33,34 Increased level of WBCs and Lymphocytes suggesting that the TME extract challenge the immune system of the animals. Liver protective outcome of TME was further investigated by histopathological study. TME at different dose levels offers liver protection, but 400 mg/kg is more effective than all other inferior doses. As demonstrated in the present study, administration of simvastatin significantly elevated serum levels of hepatic enzymes, and that representing significant hepatocellular harm. Thus, our study confirmed the hepatoprotective potential of Trichosanthes dioica Roxb against simvastatin induced liver toxicity in rats. In fact, activity of Trichosanthes dioica Roxb is quite like silymarin, as reference hepatoprotective agent.

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CONFLICT OF INTEREST

No conflict of interest associated with this research work.

ABBREVIATIONS

SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; ALP: Alkaline phosphatase; SEM: structural equation modeling; SIM: Simvastatin; SLY: Silymarin.

REFERENCES


**GRAPHICAL ABSTRACT**

**SUMMARY**

- Hepatoprotective potential methanolic fruit extract of *Trichosanthes dioica* Roxb was estimated along with its effect on biochemical and haematological indices in hepatotoxicity induced by simvastatin in rats. The results demonstrate the alterations in elevated levels of SGOT, SGPT, ALP, total bilirubin and restored the total protein and albumin level. The histological study showed regeneration of hepatocytes around central vein with near normal liver architecture revealing hepatoprotective effect of fruit extract.

**ABOUT AUTHORS**

Ramesh Kumar Gupta: Asst. Professor, Sherwood College of Pharmacy, Barabanki.